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USDA-ARS National Research Action Plan for Development of Technologies for Management and Suppression of the Gypsy Moth, *Lymantria dispar*

In cooperation with —
USDA Forest Service
USDA Animal and Plant Health Inspection Service
USDA Cooperative State Research Service and the
State Agricultural Experiment Stations
USDA Extension Service

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Preface

The primary aim of the ARS gypsy moth program is to provide the necessary research - through a cohesive team effort - that will lead to environmentally, economically, and publicly acceptable technologies for management of gypsy moth populations. These technologies will support the implementation of State and Federal research, action and regulatory programs.

The research action plan contained herein details the ARS fundamental and applied gypsy moth program, including its cooperative efforts with Federal and State agencies, the State Agricultural Experiment Stations, and the private sector. A unified team effort is needed in order to help solve the specific problems related to this devastating insect pest. This requires the development of a comprehensive national plan that clearly defines program goals and objectives, identifies each project's relevance and fit, identifies activities and time frames needed to reach objectives. This plan can serve as a basis for full participation of ARS scientists and its cooperators.

The research action plan will help provide (a) program focus, (b) a basis for monitoring and evaluating program progress, (c) a basis for developing budget estimates and allocating resources, (d) responsiveness to the technology and problem-solving needs of action agencies, (e) identification of technology transfer opportunities, and (f) a framework for a unified effort. Additionally, the research action plan will provide an important foundation for program strengthening and expansion, and coordination, both intra- and extra-murally. The plan is a dynamic one and progress in reaching its goals will be reviewed annually; the plan will be revised by the addition of published supplements and abstracts detailing progress. As deemed appropriate and necessary, workshops will be scheduled periodically to foster communication, coordination, and to examine priorities; participants are expected to play a significant role in redefining essential activities when necessary.

Over the past few years, several noteworthy accomplishments have been achieved. The abnormal performance syndrome (APS), a problem with so-called "straggling" larvae during mass rearing, appears to have been eliminated at the APHIS Otis Air National Guard Base facility in Massachusetts. Improved strains of the gypsy moth nuclear polyhedrosis virus and *in vitro* production technology developed by ARS scientists have been transferred to American Cyanamid, Inc., Princeton, New Jersey. Continuing efforts are being made to enhance virus efficacy with a group of chemical compounds, the fluorescent brighteners, that increase virus efficacy many fold. Urban management strategies are currently being formulated in a multi-pronged approach utilizing the results from ARS research on gypsy moth biology coupled with selective management tactics and decision and systems analysis. It is expected that exciting new advances will continue to be achieved over the next few years through a concerted team approach and effort.

The National Program Staff expresses its appreciation to all those individuals who contributed to this research action plan. Special appreciation is accorded to Dr. Edward M. Dougherty of the Insect Biocontrol Laboratory, Beltsville, Maryland, who was assigned to the task of coordinating input and helping to compile the various components of the plan.

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Foreword

In the spring of 1992, Dr. Robert M. Faust of the ARS National Program Staff requested that a research action plan for development of technologies for management and suppression of the gypsy moth be developed for use by the Agency and its cooperators. A key program function is to ensure that ARS gypsy moth research projects collectively form an effective response to this serious insect problem, and thus conform to the Agency's 6-year strategic and implementation program plan. I was assigned the task of helping to coordinate the development of the plan.

The various projects comprising the overall ARS gypsy moth research program are conducted within 7 ARS management units at 6 locations and involve close to two dozen scientists as of October 1992. The national research action plan detailed in this document is divided into four main research action areas: (A) Fundamental Biology; (B) Biological Control; (C) Detection and Technology; and (D) Modeling and Systems. Also included are other sections describing the overall mission of the national gypsy moth program, agency-by-agency responsibilities, a historical perspective of gypsy moth control, and an overview of current ARS research, laboratory-by-laboratory, including research accomplishments, research objectives, significance, constraints, cooperators, potential uses of research findings, and thoughts on research needs.

The national gypsy moth research action plan contained in this document is the result of input from many scientists within the various laboratories involved with gypsy moth research. I would like to express my appreciation to all the participants in helping to develop this national research action plan. I am especially indebted to Dr. Robert M. Faust of the National Program Staff for giving me this opportunity to be involved in this important activity and for his guidance and direction, to Dr. Roger Fuester, ARS, Newark, Delaware, for his help and valuable advice in the development of the historical perspective section of the document, and to Rosemary Ershaw for her valuable help in the overall project coordination as well as producing the document drafts.

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I. EXECUTIVE SUMMARY

The gypsy moth, Lymantria dispar (L.), is one of the most important forest and shade tree pest in the United States. Since its introduction into New England over 100 years ago, it has defoliated over 50 million acres of woodlands in the northeastern states and imperils other regions as it continues to spread. Unlike many forest pests, the gypsy moth has great impact in suburban and residential areas, where effective, economical, environmentally compatible and publicly-acceptable methods of control are needed.

The mission objectives of the ARS gypsy moth research program are two-fold: (1) to develop feasible means of protecting high value trees in non-forest environments, and (2) to support the activities of Federal and State action agencies. To accomplish these objectives ARS undertakes a vigorous program of basic and applied research that compliments other gypsy moth Service programs within the Department of Agriculture (Animal and Plant Health Inspection Service, Cooperative State Research Service, Extension Service, and Forest Service), as well as those programs of the State Agricultural Experiment Stations, State action agencies, and the private sector.

ARS has sustained an active gypsy moth research program since 1972, when it redirected $1 million for research on this pest. In 1989 six high-priority gypsy moth research areas were identified by the ARS: (1) improved methods of mass rearing, (2) cooperation with APHIS in the development of a sterile male technique, (3) increased efficacy of the gypsy moth nuclear polyhedrosis virus (NPV), (4) low-population density intervention, (5) urban management strategies, and (6) containment strategies. Steps to provide accelerated research activities have been implemented in the areas of mass rearing technology, sterile male technique, NPV research, and development of urban management strategies. In FY 1993, approximately $1.9 million were appropriated to ARS for in-house research on gypsy moth. These funds are utilized to support the variety of projects with a total of 7.9 scientist years at Beltsville, MD, Washington, DC, Otis ANGB, MA, Newark, DE, Seoul, Korea, and Montpellier, France. As a result of this action plan, the ARS gypsy moth research program is now categorized into four broad areas:

- Fundamental biology, particularly studies on biosystematics, neurohormones, diapause, reproduction, behavioral physiology, genetics, and ecology.
- Biological methods of control, with emphasis on natural enemies (parasites, predators, and pathogens).
- Detection and survey technology.
- Development of management programs and systems for use in suburban/urban parks and residential areas.

The research action plan contained herein provides the framework and 5-year program of research on gypsy moth within the ARS and details its extramural linkages. The plan is to be considered a dynamic one and will be modified periodically to reflect goals achieved; as needed, priorities and goals will be redefined with resources appropriated to meet priorities and goals as they can be made available.

II. INTRODUCTION

The gypsy moth, Lymantria dispar (L.), is unquestionably one of the most important forest and shade tree pests in the United States. Imported in 1868 by Leopold Trouvelot, a French naturalist, in an attempt to commence a silk industry in order to supply New England textile mills, some of the caterpillars escaped, and the moth became established in Medford, Massachusetts. Although Trouvelot notified local officials that a potential plant pest problem existed, no attempts were made to eradicate the moth at that time. This infestation increased and spread gradually, until in 1889, the insect was so numerous and destructive that it became a concern to the public. In the following year, the Massachusetts Legislature appropriated $25,000 for field operations to control the pest, and in 1891, an eradication program utilizing egg mass destruction, burning of infested vegetation, tree banding, and spraying with inorganic insecticides was implemented. This program, conducted from 1891 to 1900 was so successful in reducing the infestation that the project unfortunately was abandoned. Gypsy moth populations again increased rapidly over the next five years, with infestations moving to adjoining states.

The gypsy moth has now spread throughout the northeastern U.S., and is permanently established in all or parts of 16 states. Most recent infestations include portions of Ohio and North Carolina. The gypsy moth has caused tree defoliation on over 52 million acres since 1924, with 62 percent of that total, or 38.7 million acres occurring in the 1980's. Single year defoliation records were reported in 1980 (5.1 million acres) and 1981 (12.8 million acres). There were 4,152,000 acres defoliated in 1991. Moreover, an increase in moth distribution along the leading edge of the infested areas average about 14 million acres per year, indicating that the problem likely will continue to increase in scope.

More recently, an introduction of the Asian gypsy moth (AGM) into North America could pose new and unique problems with which to contend. The AGM was identified in Vancouver, British Columbia, as well as Tacoma, WA and Portland, OR. This pest successfully "hitchhiked" on grain ships infested in eastern Asia that later made calls in North American ports. The AGM could pose a potentially greater threat than the North American gypsy moth (NAGM) due to the ability of the female to fly. If this defoliator of hardwoods and conifers should successfully establish itself in North America, distribution among the vast forest tracts and wooded cities could be swift, based on the flight characteristics of this newly emerging related pest.

Although the gypsy moth is a well-known forest pest, it has a great impact in suburban/urban areas by causing substantial defoliation, tree mortality, and distress to homeowners. There is public demand for effective means of controlling this pest which are safe, both from a human health and an environmental viewpoint.

III. INTERAGENCY MISSION OF THE NATIONAL GYPSY MOTH PROGRAM

Agency Responsibilities

The Forest Service has been designated as Lead Agency in the Departmental gypsy moth program. Responsibilities of individual Agencies and cooperative interactions with ARS are summarized below.

Forest Service. The Forest Service (FS) serves as lead agency for Departmental gypsy moth program activities. Within the generally infested area, the Forest Service has direct responsibility for gypsy moth survey and control on Federal lands, and cooperatively with the States on non-Federal lands. Forest Service research is directed at developing integrated systems for gypsy moth control under forest and wildland/urban interface conditions, emphasizing safe, cost-effective practices that prevent populations from increasing above innocuous levels and suppress existing outbreaks. Technical assistance is provided to State agencies within the generally infested area. Pilot tests are performed to improve gypsy moth control and damage reduction practices. Financial assistance is provided to generally-infested and newly-infested States to aid in suppressing gypsy moth populations.

The Forest Pest Management Division of FS supports two major programs on gypsy moth: (1) the Federal-State Cooperative Suppression Program, and (2) the Appalachian Integrated Pest Management Project (AIPM) which will be completed by the latter part of 1992.

The Federal-State Cooperative Suppression Program targets for suppression high populations (250 egg masses [EM]/acre) of the pest, and during each of the past two years, about 800,000 acres were treated. In most years, acreage requiring treatment far exceeds available resources, so the long term goal is to maintain populations below the 250 EM/acre level. Development of better formulations of Bacillus thuringensis (Bt) so that treatments in successive years are not needed, knowledge of the effects of BT on non-target species, and cost-effective means of producing the gypsy moth nucleopolyhedrosis virus are pressing needs.

The 5-year AIPM Project was established in 1987 for the purpose of (1) minimizing gypsy moth spread and impact in the project area, (2) developing a prototype IPM system that can be used anywhere in the U.S., (3) evaluating the use of intervention activities for management of isolated infestations within the project area, and (4) assessing the feasibility of a coordinated county, State and Federal gypsy moth program. Several ARS scientists have served on a AIPM Technical Working Group.

Animal and Plant Health Inspection Service. Regulatory aspects of the Department's gypsy moth program are administered by APHIS. APHIS coordinates, through appropriate State agencies, a national survey program to detect isolated gypsy moth infestations. Methods development and technology transfer activities are conducted to improve gypsy moth eradication and quarantine practices. A national regulatory program is maintained to prevent the artificial long-range spread of the gypsy moth, and limited financial

assistance is provided to previously uninfested States outside the generally infested area to eradicate isolated gypsy moth infestations. APHIS also provides clearance services for exotic organisms imported for biological control research.

Through a Memorandum of Understanding between APHIS and FS, the FS has responsibility for eradication of gypsy moth infestations greater than one square mile and APHIS has responsibility for eradicating smaller infestations. At the APHIS Methods Development Center, Otis Air National Guard Base, Massachusetts, major emphasis is placed on the development of sterile male techniques and the improvement of rearing as well as monitoring techniques. ARS has cooperated with APHIS in addressing the so-called "straggling" problem in the rearing of gypsy moth. ARS provides assistance in the form of overseas collection and quarantine handling of exotic natural enemies for the Otis facility which serves as a quarantine unit for Asian gypsy moth investigations in the U.S.

Agricultural Research Service. Research in the ARS is aimed at developing the means to protect high-value trees for homeowners, communities, parks and other non-forest environments; and in developing technology in support of the activities of action agencies.

The primary ARS gypsy moth research activities include (1) fundamental biology (systematics, physiology, reproduction, and sterility), (2) biological control suppression methods (microbial agents, parasites and predators), (3) detection and survey technology, and (4) management systems for use in urban/suburban parks.

ARS also funds several extramural research projects with local and State governments as well as colleges and universities. For 10 years, ARS served as a cooperating agency to the Cooperative State Research Service (CSRS) Regional Project NE-143, "The Gypsy Moth and Its Natural Enemies: Behavioral and Population Determinants," which expired September 1992. It is anticipated that ARS will serve in the same capacity on the new replacement CSRS Regional Project NC-164, slated for approval May 1993. ARS has also provided cultures of exotic gypsy moth parasites to a number of State agencies (Pennsylvania Department of Environmental Resources, New Jersey Department of Agriculture, and others) for use in biological control purposes.

Cooperative State Research Service Including the State Agricultural Experiment Stations (SAES) and Other Universities, Colleges and State Organizations. The CSRS plans research in cooperation with State Agricultural Experiment Stations; Forestry Schools, and other cooperators; and administers a research grants program and a coordinated regional research program on gypsy moth.

A Federal-State Cooperative Suppression Program provides for cost-sharing of Federal, State, and local (county or municipal) funds to control high populations of gypsy moth. Intervention policies are developed by managers directing the individual State suppression programs. Standardization of survey methods (especially in urban areas), cost-benefit concerns, and ready-to-use informational packages are high priority considerations for State suppression programs.

Extension Service. The Extension Service (ES) coordinates an information and education program carried out by Departmental and appropriate State agencies on gypsy moth and gypsy moth management practices.

IV. AN HISTORICAL PERSPECTIVE OF GYPSY MOTH CONTROL

ARS and its predecessor agencies have been involved with gypsy moth control since 1906, when the Department's Bureau of Entomology and the State of Massachusetts jointly financed importations of natural enemies of the gypsy moth in hopes of checking the spread, or eliminating the pest from the U.S. The development of DDT during the 1940's, along with a resurgence of gypsy moth during the early 1950's, led to plans for a long-range program to address the gypsy moth problem in the U.S.

A contingency plan of eradicating all infestations back to a barrier zone along the eastern New York border was set up in case the primary objective of total eradication proved unfeasible. Over 2 million acres were sprayed with DDT in 1957; in the following year, only 125 acres of forested areas were defoliated in the treated region. However, increased environmental concerns about residues of persistent pesticides resulted in a decision to phase out DDT, and carbaryl, though not as effective as DDT, became the insecticide of choice. In 1959, defoliation by the gypsy moth was on the increase again, diminished slightly in 1966-68, but then increased significantly throughout the Northeast in 1969. This outbreak gave impetus to the support of strong research programs aimed at developing effective control technologies.

In 1971, the USDA began its Accelerated Program for research and development on the gypsy moth, with increased base funding and resources for research and development to both ARS and FS. In FY 1972, Congress appropriated to ARS $1 million for research on gypsy moth, and the overall level of ARS resources devoted to gypsy moth research has approximated that ever since. Major ARS research activities that were initiated included (1) development and evaluation of a synthetic sex attractant, disparlure, (2) an increase in foreign exploration for parasites and predators, and (3) developmental research on microbial agents causing diseases of the gypsy moth.

Between 1971 and 1973, gypsy moth infestations continued to worsen, with severe outbreaks being recorded in Connecticut, New York, New Jersey, and Pennsylvania. Also, problems with the Douglas-fir tussock moth in the Pacific Northwest and the southern pine beetle in the Southern U.S. created widespread concern, and in 1975, the USDA initiated a Combined Forest Pest Research and Development Program on all three pests. The program lasted four years (1975-78) and had a broad range of objectives: (1) development of better methods for predicting pest population trends, (2) measuring and predicting impact of the pests, (3) registration of nuclear polyhedrosis virus (NPV), (4) development of formulations and application technology for microbial and chemical insecticides, (5) demonstration of potential for use of disparlure in containment, suppression, and possible eradication; (6) screening, laboratory evaluation, and field testing of new chemical insecticides; (7) evaluation of available and newly introduced parasites and predators, (8) evaluation of potential of sterile male techniques for suppression or eradication, and (9) development of a mass-rearing capability for program support.

Late in 1981, a strategic planning session among scientists from ARS, CSRS, FS, APHIS, several State Agricultural Experiment Stations, and private industry resulted in the development of a USDA Comprehensive Gypsy Moth Research Plan in 1982. In 1987, an intensive review of the ARS gypsy moth program was followed by a planning workshop in 1989. USDA Interagency Gypsy Moth Research Reviews are held annually. The Northeastern Forest Experiment Station (FS) released a "Gypsy Moth Research and Development Program/Research Plan 1991-1995, Planning Document and Activity Schedule" in June 1992. Since the commencement of the ARS Accelerated Program, research on gypsy moth has been conducted by at least 11 ARS research management units at some six locations, two of which have been overseas.

V. RESEARCH AREAS AND PLANS

PARTICIPANT CODES

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RESEARCH AREA A

Fundamental Biology

RESEARCH AREA A - Fundamental Biology

	Research Approaches	Year 1	Year 2	Year 3	Year 4	Year 5
		Activities				
A.1.	Biosystematics (also, see B.2.d, B.2.h, B.2.j, B.2.k, B.2.l, B.2.o, B.2.p, C.1.a)					
A.1.a	Construction of Lymantriidae catalog.	Construct catalog and database. RWP	Construct catalog and database.	Publish catalog.	--	--
A.1.b	Investigate systematics of world species of gypsy moth complex.	Assemble material and data from many sources and analyze for morphological and other differences. DCF	Continue gathering and analyzing data and evaluate male and female genitalia as character systems; determine number of species and correct nomenclature.	Publish results.	--	--
A.2.	Genetics (also, see A.3.g, A.3.h, A.3.k, A.3.p, B.1.l, B.1.m)					
A.2.a	Develop understanding of genetic factors involved in regulation of diapause.	Determine mode of inheritance of diapause. RAB	Continue study of mode of inheritance of diapause.	Isolate and purify diapause associated proteins.	Clone diapause associated genes and determine cDNA sequences.	Transfer technology to users for improving insect rearing and control technology.
A.2.b	Cell & tissue viral transformation.	Characterize transformed cell lines; determine likely cells that can be transformed. EMD	Determine if gypsy moth can be transformed; determine if viral integration occurs.	Make libraries of transformed cells; determine site of integration.	Make vector from knowledge of year 3.	Transform insect tissue with foreign gene.
A.2.c	Compare reproductive biology of AGM with that of NAGM, & insects from field collected egg masses.	Descriptive biology - no. of sperm bundles, time of mating, multiple matings, fecundity & fertility; set up intercross. FIP	Fertility & fecundity of hybrid & 1st generation backcross.	Fertility & fecundity of 2nd & third generations.	--	--
A.2.d	Reproductive biology of colonized and wild gypsy moths; factors contributing to problems in rearing. (APS)	Effect of female age at time of mating; frequency of multiple mating in both sexes and effect of container environment upon oviposition. FIP	Determine effect of various climate variables on adult longevity and mating success.	Determine effect of crowding upon reproduction.	--	--

RESEARCH AREA A - Continued

	Research Approaches	Activities: Year 1	Year 2	Year 3	Year 4	Year 5
A.2.e	Assess F_1 sterility as a control strategy.	Complete studies on sperm transfer and mating fitness in the laboratory, including female mate selection when offered both F_1 and nonirradiated males. FIP	If laboratory studies are promising, conduct field interaction studies including multiple mating of females.	Complete interaction studies in the field.	--	--
A.3.	Physiology and Biochemistry (also, see C.1.a)					
A.3.a	Determine endocrine and neuroendocrine factors involved in embryonic development and diapause.	Determine rates of respiratory activity during embryogenesis and diapause. RAB	Establish ecdysteroid & prothoracicotropic hormone titers during embryonic development and diapause.	Determine role of juvenile hormone in regulation of diapause.	Investigate role of diapause hormone or other inhibitory factors.	Develop a conceptual model of endocrine regulation of late embryonic diapause.
A.3.b	Develop practical methods for preventing or precociously terminating diapause.	Test previously defined methods for disrupting diapause in other insects. RAB	Investigate use of hormones, anti-hormones and mimics for disruption of diapause.	Refine methods for practical disruption of diapause.	Transfer technology for disruption of gypsy moth (GM) diapause to other species.	Synthesize information and develop theory for practical manipulation of insect diapause.
A.3.c	Improve insect rearing and biocontrol technology by application of insect hormones and/or their analogues.	Determine effects of JH, anti-JH and ecdysteroid agonists on GM growth and development. RAB	Determine optimal mode of application of exogenous hormones.	Refine methods of application.	Conduct lab and small scale field trials.	Apply knowledge toward improved rearing/production of biocontrol agents or hormonal pest control.
A.3.d	Isolate hindgut ecdysiotropic peptide(s), sequence, and produce antibodies to the peptide(s).	Rear insects; Collect hindguts; Utilize HPLC and bioassay to purify ecdysiotropin(s). DBG	Sequence peptide and produce antibodies to peptide.	Utilize immunocytochemistry to locate sites of synthesis and sites of action.	Isolate receptor; design analogues to interfere with action of ecdysiotropin.	--

RESEARCH AREA A - Continued

	Research Approaches	Activities: Year 1	Year 2	Year 3	Year 4	Year 5
A.3.e	Determine physiological roles(s) of the hindgut ecdysiotropin(s).	Compare activity at different times of day. DBG	Culture hindgut tissue to determine if and when release into hemolymph occurs.	Determine the effect of brain and other neuroendocrine organs on synthesis and release of ecdysiotropins.	Determine mode of action of ecdysiotropins and interaction with other regulatory molecules in stimulating the production of molting hormone.	--
A.3.f	Isolate, purify and sequence PTTH.	Utilize in vitro and in vivo PTTH assays in initial isolation steps for egg and larval gypsy moth PTTH. TJK, EPM	Develop the final purification steps for these PTTH's.	Isolate and sequence the various gypsy moth PTTH's.	Characterize their activity in vitro and in vivo.	Develop photoaffinity labelled probes for isolation of the PTTH receptor.
A.3.g	Clone and express the PTTH gene.	Develop PCR screening methods for the gypsy moth PTTH gene. TJK, EPM	Screen for and isolate the gypsy moth PTTH gene using Bombyx PTTH based primers.	Vector express the gypsy moth PTTH gene.	Test the expressed PTTH, constructs and the natural, purified PTTH, if available, in in vitro and in vivo bioassays.	Characterize specific regions essential for PTTH bioactivity by site-directed mutagenesis.
A.3.h	Develop JH and vitellogenesis systems.	Establish a radioimmunoassay for gypsy moth JH. TJK	Correlate JH titers with egg diapause and vitellogenins synthesis; verify JH3 as the natural JH of gypsy moth.	Develop PCR screening for the gypsy moth vitellogenin gene based on conserved N-terminal sequence data from other species.	Screen for and isolate the gypsy moth vitellogenin gene and its 5' regulatory regions.	Sequence the 5' region and identify JH regulatory sequences.
A.3.i	Testis ecdysiotropin. (TE)	Determination of structure. MJL	Prep. of antibodies to TE; start structure-function studies.	Immunocytochemistry; continue structure-function studies.	RIA and ELISA developed.	Modes of action.
A.3.j	Growth factors.	Isolation of growth factors. MJL	Determination of molecular properties.	Structure determination and structure-function studies.	Modes of cell interaction.	Roles in regulating insect growth and reproduction.

RESEARCH AREA A - Continued

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
A.3.k	Neuropeptide isolation and characterization; molecular genetics.	Isolate cerebral PTTH and begin isolation of non-cerebral ecdysiotropin (NCE). EPM	Sequence PTTH, isolate NCE; begin studies on PTTH gene.	Identify PTTH gene; insert into baculovirus vector system, deduce pre-& pro-PTTH structure; sequence NCE.	Identify NCE gene and deduce pre-& pro-structure of neuropeptide.	Insert NCE gene into baculovirus vector system; use synthetic PTTH and NCE for physiological studies.
A.3.l	Structure-function studies.	Construct and test analogs of PBAN in vivo. EPM	Construct and test analogs of PTTH; start receptor and MOA studies.	Identify receptors for PBAN and/or PTTH; continue MOA studies.	Synthesize and test analogs and binding inhibitors; isolate receptors.	Explore analogs/ inhibitors as control agents.
A.3.m	Enzyme analysis and characterization.	Refine sub-cellular fractionation and membrane preparation methods; develop assays. EPM	Characterize enzymes; conduct kinetic studies.	Use characterized enzymes in studies on PTTH & PBAN processing and metabolism.	Determine effect of enzyme inhibition in vitro and in vivo.	Explore enzyme inhibitors as control agents.
A.3.n	Developmental studies.	Produce anti-PBAN antibody; develop ELISA and RIA; test analogs of PBAN; produce anti-PTTH antibody. EPM	Use anti-PBAN antibody to titer PBAN during development and mate-calling; use anti-PTTH antibody to titer PTTH during development (develop ELISA and RIA).	Initiate studies on PTTH and PBAN biosynthesis and processing.	Continue PTTH and PBAN processing studies; initiate processing studies with NCE.	Screen for processing inhibitors; identify enzymes involved; synthesize processing inhibitors; examine in vivo interaction between PTTH and NCE.
A.3.o	Determine regulation of sex pheromone production in gypsy moth female.	Determine mechanisms of pheromone inhibition after mating and neural innervation of pheromone gland. AKR	Determine mode of PBAN action in virgin females.	Continue to study role of PBAN in activating pheromone synthesis; determine pattern of pheromone production in various temperature cycles.	Study mechanism of the inhibition of pheromone production by high temperature; initiate development of PBAN antagonists.	Continue study of antagonists that can disrupt pheromone production.

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
A.3.p	Determine molecular basis of sperm release and maturation in gypsy moth males.	Test effects of pharmacological agents on sperm release; complete analysis of proteins involved in mechanism of sperm release. AKR	Identify proteins which show daily changes in the rate of synthesis and state of phosphorylation.	Sequence proteins of interest and study their role in sperm release and maturation.	Conduct mRNA- cDNA subtractive hybridization to identify genes with daily rhythm of transcription/translation; clone genes coding for proteins sequenced during year 3.	Produce antisense mRNA for genes that are involved in sperm release mechanism; attempt to disrupt sperm release using antisense mRNA.
A.4.	Behavior (also, see C.1.a)					
A.4.a	Characterize larval responses to selected control tactics.	Analyze larval responses to barrier bands using video equipment (lab). REW	Analyze larval responses to barrier bands in the field, video studies of larval behavior as modified by NPV and/or B.t.	Field studies of behavior as modified by NPV, video studies of behavior as affected by tactic/natural enemies (B.t., microsporidia, CPV, parasites).	Further lab/field studies as in year 3.	Continued as needed.
A.4.b	Behavior studies of Asian gypsy moth.	Determine female flight behavior in Asian gypsy moth. PWS	--	Investigate evidence of diminished dispersal in neonate larvae.	--	--
A.4.c	Investigate gypsy moth (GM) defense secretion.	Identify "defense" secretion in larval GM. PWS	Determine function.	Determine effects on natural enemies.	Possible manipulation of populations.	Same as year 4.
A.5.	Ecology					
A.5.a	Population dynamics and epidemiology of applied microbials.	Evaluate second wave effects of applied NPV in microbial control study plots. REW	Using genetically-identifiable strains, determine interactions of applied NPV with natural NPV in replicated virus/B.t. study.	Same as yr 2, if needed.	Same as yr 3, but in pilot study of NPV application to parks.	Same as yr 4, if needed.

RESEARCH AREA B

Biological Control

RESEARCH AREA B - Biological Control

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
B.1.	Pathogens					
B.1.a	Fluorescent brightener, mode of action.	Define rate of entry of virus via oral & hemocoel. EMD	Measure effect of fluorescent brightener on entry rates.	Measure early events in permissive midgut.	--	--
B.1.b	Heterologous virus mode of entry.	Define ability of AcNPV to enter L. dispar. EMD	Determine kinetics of entry.	Measure early events in midgut tissue.	--	--
B.1.c	Begin analysis of virus replication in current media.	Test revised formulations to improve & stabilize virus replication. JLV	--	--	--	--
B.1.d	Develop parameters for optimizing cell growth in 1-3 liter suspensions/virus replication.	Test media formulations for virus replication in suspension. JLV	Develop parameters for optimizing virus production in suspension.	--	--	--
B.1.e	Effect of fluorescent brightener/NPV on gypsy moth gut.	Obtain MAB to antigen of LdMNPV envelope. JRA	Use MAB to locate viral DNA of LdMNPV in gut.	--	--	--
B.1.f	Determine effects of plant materials upon the gypsy moth and its NPV.	Develop information on different host plant chemicals upon growth and development of gypsy moth larvae and its NPV. MS	Select most promising materials for further study.	Assess potential of these materials in greenhouse and/or small-scale field tests.	--	--

RESEARCH AREA B - Continued

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
B.1.g	Determine effects of cytoplasmic polyhedrosis virus (CPV) upon gypsy moths.	Develop information on the effects(s) of CPV upon larval growth and the biological activity of the virus upon gypsy moth larvae. MS	Determine interaction of CPV with LdMNPV.	Asess potential of CPV as a microbial control agent in greenhouse and/or small scale field tests.	--	--
B.1.h	Determine effects of stilbene optical brighteners upon efficacy of gypsy moth NPV and other entomopathogenic viruses.	Develop information on the effect of selected optical brighteners upon the gypsy moth and its NPV. MS	Assess potential of selected optical brighteners as adjuvants for LdMNPV in small-scale field tests; develop information on the effect of selected brighteners upon gypsy moth CPV and other viruses against the gypsy moth.	Develop parameters for field applications; develop information on the effects of stilbene optical brighteners upon other insects and other insect pathogenic viruses.	--	--
B.1.i	Determine mechanisms of UV protection.	Develop information on different groups of chemicals as UV radiation protectants. MS	Select most promising materials for further studies; initiate studies on radical scavengers.	Assess potential of these materials for greenhouse and/or small-scale field tests.	--	--
B.1.j	Determine susceptibility of gypsy moth larvae to different insect pathogenic viruses.	Develop information on the activities of different insect pathogenic viruses upon gypsy moth larvae; initiate <u>in vivo</u> selection and passage studies. MS	Continue selection and passage of the most promising viruses; compare these adapted viruses to the LdMNPV in standard bioassays.	Assess potential of these viruses as microbial control agents in greenhouse and/or small scale field tests.	--	--

RESEARCH AREA B - Continued

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
B.1.k	In vivo selection of more potent NPV biotypes.	Initiate in vivo selection of LdMNPV using time (early kill, LT_{50}) and virus concentration (LC_{50}, LC_{90}) as selectable attributes; select for most active and least active biotypes. MS	Continue selection until activities are stabilized; initiate in vitro propagation of these biotypes.	Assess potential of the most active biotypes as microbial control agents in greenhouse and/or small-scale field tests; determine genetic and biochemical characteristics of the most active and least active biotypes.	--	--
B.1.l	Chemical selection of more potent NPV biotypes.	Initiate selection of LdMNPV using known DNA intercalators. MS	Continue selection until activities are stabilized.	Assess potential of most active biotypes as microbial control agents in greenhouse and/or small scale field tests; assess genetic and biochemical changes during selection.	--	--
B.1.m	Quantitate efficacy of aerial B.t. applications in parks and residential areas.	Develop methodology necessary to accurately estimate larval mortality due to aerial applications of B.t. RLR	Determine relationship between spray droplet deposition and biological efficacy.	Collect data on efficacy of B.t. from a variety of gypsy moth populations and densities in parks and woodlots.	Collect data on efficacy of B.t. from residential areas treated during state and county suppression programs.	Continue as in years 3 and 4 as necessary.
B.1.n	Evaluate potential of nutrient-based phagostimulants for increasing efficacy of microbials.	Measure effects of at least 5 commercial phagostimulants on food consumption. RLR	Establish relationships between B.t. dosage and effects on feeding behavior.	Conduct laboratory studies on the interactive effects of a phagostimulant, B.t., and host-plant material.	Determine the changes in efficacy of B.t. and NPV applied with a phagostimulant on host plants.	Conduct field trials if lab and greenhouse results show adequate promise.

RESEARCH AREA B - Continued

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
B.1.o	Evaluate alternative control materials using ground based sprays.	Evaluate vegetable oils as ovicides; evaluate NPV and potentiator against older larvae on individual trees. REW	Continue NPV studies; evaluate different B.t. formulations and insecticidal soaps as individual tree treatments.	Continue B.t./soap studies and evaluate parasitic nematodes as individual tree treatments	Continue nematode studies.	Reassess state-of-art, design individual tree treatment studies accordingly.
B.1.p	Evaluate the establishment of introduced microbes in the mid-Atlantic states.	Evaluate the establishment of introduced microsporidia. REW	Same as year 1, obtain regulatory permission to release CPV.	Release CPV.	Evaluate the establishment of introduced CPV.	Same as year 4.
B.1.q	Develop cost-effective NPV/potentiator doses/concentration for urban environs.	Evaluate NPV+ potentiator in one or more field locations (single trees, small plots, and/or aerial applications). REW	Evaluate in-vitro produced NPV in field locations (single trees, small plots, and/or aerial applications).	Evaluate commercial formulations of NPV/ potentiator combinations.	Same as year 3, if needed.	Same as year 4, if needed.
B.1.r	Quantitatively evaluate efficacy of NPV in urban parks.	Monitor/survey candidate field plots. REW	Replicated evaluation of improved NPV product against B.t. standard; survey candidate parks.	Additional replicated studies; quantitative assessment of NPV in parks.	Quantitatively evaluate relationship between NPV spray deposit, larval mortality and defoliation.	Validate year 4 findings.

RESEARCH AREA B - Continued

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
B.2	Parasites and Predators					
B.2.a	Determine the role of parasites and predators in population dynamics of gypsy moth.	Obtain census and mortality data during latent (=innocuous) phase of population cycle in study plots. RWF	Obtain census and mortality data during latent/progradation (=release) phase of population cycle.	Obtain census and mortality data during progradation phase of population cycle.	Identify key natural enemies involved in gypsy moth regulation and gaps in natural enemy complex.	Determine potential methodologies for exploiting key natural enemies during latent or progradation phase of population cycle.
B.2.b	Importation and release of promising natural enemies.	Quarantine evaluation, Blepharipa schineri; release of previously approved species in Southern states. RWF	Quarantine evaluation, Blepharipa schineri, Ceranthia samarensis, & new biotic agents; release of previously approved species in Southern states.	Quarantine evaluation, Ceranthia samarensis & new biotic agents; release of Blepharipa schineri and other approved species.	Quarantine evaluation of new biotic agents; release of Biepharipa schineri, Ceranthia samarensis and other approved species.	Complete releases of the most promising species.
B.2.c	Evaluation of natural enemy buildup, dispersal, and effectiveness.	Continue evaluation of Coccygomimus disparis on DelMarVa peninsula. RWF	Complete evaluation of Coccygomimus dispar; begin recovery attempts in Southern states.	Continue evaluations in Southern states; begin recovery attempts for B. schineri.	Complete evaluations in Southern states; continue evaluation of B. schineri; begin recovery attempts for C. samarensis & other species.	Document successful establishments and target successes for further evaluation.
B.2.d	Collection and importation of L. dispar natural enemies.	Collect and ship exotic natural enemies in southern France. FH & LK	Same as year 1, but in Italy and Algeria.	Same as year 1, but in eastern Europe.	Same as year 1, but in central Asia.	Same as year 1, but in central Asia.

RESEARCH AREA B - Continued

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
B.2.e	Alternate host requirements by multivoltine L. dispar (LD) parasitoids.	Determine occurence of parasitoids in late exposure of experimental cohorts of LD and in collections of other pests. FH & LK	Study host acceptance and host suitability of LD and alternative hosts for multivoltine LD parasitoids.	Determine potential mechanisms involved in natural enemy host finding.	Evaluate search rate and heritability of search rates in multivoltine LD parasitoids.	Determine factors affecting interactions of host finding mechanisms, host, and host plants.
B.2.f	Effect of host density on parasitization by C. separata and P. inconspicus.	Measure host abundance and parasitization by C. separata and P. inconspicus at different sites. Check for density-dependent responses. FH & LK	Same as year 1.	Same as year 1.	Same as year 1.	Same as year 1.
B.2.g	Host attack behavior and bioecology of B. pratensis and B. schineri.	Measure host abundance and parasitization by B. pratensis and B. schineri at different sites. FH & LK	Study of host acceptance and host suitability of L. dispar for B. pratensis and B. schineri.	Determine potential mechanisms involved in natural enemy host finding.	Evaluate search rate and heritability of search rate in B. pratensis and B. schineri.	Determine factors affecting interactions of host finding mechanisms, hosts, and host plants.
B.2.h	Korean parasite survey.	Season-long survey collections at south coast area sites of South Korea. RWP	--	--	--	--
B.2.i	Effect of ecology on parasitism.	Alternative host studies and plant community influences on parasitism of gypsy moth in South Korea. RWP	--	--	--	--

RESEARCH AREA B - Continued

	Research Approaches	Activities: Year 1	Year 2	Year 3	Year 4	Year 5
B.2.j	Peoples Republic of China (PRC) parasite survey.	Season-long survey collections at Lioaning Province, PRC sites. RWP	--	Season-long survey collections at Upper Yangtze River, PRC area sites.	--	Season-long survey collections at other PRC area sites to be determined.
B.2.k	Russian parasite survey.	Season-long survey collections at eastern Russian sites, probably Vladivostok, Amur River Zone and other areas. RWP	--	--	--	--
B.2.l	Calosoma sp. survey.	Determine diet behavior of Calosoma; survey for Calosoma. PWS	Same as year 1, plus measure impact on GM populations.	--	--	--
B.2.m	Survey potential of new parasites.	Collect & evaluate new natural enemies. PWS	--	--	--	--
B.2.n	Compile natural enemy list of gypsy moth.	Compile & update world list of natural enemies. PWS	--	--	Publish lists.	--
B.2.o	Dasychira meridionalis survey.	Conduct survey for moth. RFWS	Expand survey to SE U.S.	Add new material to colony.	Select release sites.	--
B.2.p	D. meridionalis colonization.	Est. colony on GM diet. RFWS	Continue as in year 1.	Continue as in year 2.	Continue as in year 3.	--

RESEARCH AREA B - Continued

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
B.2.q	Parasitize D. meridionalis (DM).	--	Select GM parasites and expose to DM. FWS	Continue as in year 2	Release parasites in selected woodlots.	--
B.2.r	Evaluate the role of established and newly released parasitoids in regulating gypsy moth populations.	Assess potential and assist in identifying searches in Europe for promising parasitoids for importation. RLR	Facilitate searches in Europe.	Develop conceptional model for role of selected established parasitoids in regulating populations.	Facilitate release and establishment of unestablished parasitoids.	Estimate the relative value of established parasitoids in northern and southern climates.

RESEARCH AREA C

Detection and Survey Technology

RESEARCH AREA C - Detection and Survey Technology

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
C.1.	Semiochemicals					
C.1.a	Identify the pheromone of Asian gypsy moth and look for distinguishing chemicals in cuticular waxes.	Analyze tip extracts, lab-reared females; analyze cuticular waxes extracted from lab-reared insects. BAL	Formulate pheromone if different than (+)- disparlure; extract wild insects if characteristic chemical waxes are found.	Test dispensers in native populations; analyze waxes of trapped males.	Implement use of dispensers in detection traps; conduct analyses of Asian and North American gypsy moths.	Serve as chemical consultant for APHIS programs.
C.2.	Traps, Devices and Formulations					
C.2.a	Develop PVC/twine dispenser for use in detection traps.	Work with industry to make 15,000 PVC/twine dispensers for APHIS evaluation. BAL	Evaluate results; license patent; incorporate additional dispensers in APHIS program.	Consider replacing all detection dispensers with PVC/twine type; monitor results.	Monitor dispensers retrieved from field.	Same as year 4.
C.2.b	Develop pheromone formulations for use in mating disruption.	Apply and evaluate microbeads in field test in VA; perform dose/efficacy relationship. BAL	Based on previous year's results, compare beads and laminate flakes; begin to develop alternate formulations.	Participate in FS and APHIS mating disruption tests; evaluate new formulations.	Participate in FS and APHIS tests; pursue new formulations.	Participate in FS and APHIS tests.
C.2.c	Determine the potential usefulness of pheromone traps in gypsy moth population monitoring programs.	Determine optimum dose and trap type based on statistical consideration of trap. RLR	Relate trap catch to population density in operational units (e.g. spray blocks, parks, residential communities) as measured by other methods (e.g. egg mass counts, pupal indices, defoliation).	Examine effects of operational unit size and characteristics on trap catch.	Determine optimum trap placement and configuration within operational unit.	Validate population density estimates based on pheromone trap data by locating traps within operational units in state or county gypsy moth suppression programs.

RESEARCH AREA C - Continued

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
C.2.d	Quantify and improve the efficacy of barrier bands against gypsy moth larvae.	Determine the ability of commercially available bands to stop gypsy moth larvae in laboratory and greenhouse assays. REW	Determine the efficacy and cost-effectiveness of the most promising commercial bands in the field.	Expand the field studies to quantify the effects of bands on populations and damage on individual trees.	Examine the effects of bands of populations and damage in stands of trees.	Continue as in years 3 and 4.
C.3.	Survey/Monitoring of Gypsy Moth Populations					
C.3.a	Quantify the distribution of gypsy moth egg masses in parks, residential areas, and on individual trees.	Collect and organize a data base of egg mass distributions; obtain data from collaborators, if available. KWT	Apply appropriate statistical methods, including geostatistical analysis, to characterize distribution.	Examine the effect of gypsy moth density, period of population cycle, and habitat on distribution.	Design and conduct manipulative experiments to test hypotheses about the effects of selected factors on distribution.	Continue as in year 4.
C.3.b	Quantify gypsy moth distribution and movement throughout the canopy and on nontree substrates.	Develop methods to accurately estimate absolute numbers of larvae in the canopy and on other substrates, and to quantify larval movement. KWT	Quantify distribution of larvae on trees and other substrates in parks and on individual trees.	Quantify movement of larvae into and out of trees and other substrates in parks and on individual trees.	Determine the effects of population density and host-tree species on larval distribution and movement in parks and individual trees.	Continue as in year 4.
C.3.c	Determine the potential for burlap samples to estimate gypsy moth population density and rates of parasitism and disease.	Relate burlap counts to absolute population density estimates (frass counts) in parks and on individual trees. KWT	Determine effects of gypsy moth population density on burlap counts.	Determine relationships between parasitism and disease indices based on burlap samples and actual levels based on other sampling methods.	Evaluate other types of artificial refugia, such as artificial bark flaps and materials other than burlap.	Continue as in year 4.

RESEARCH AREA C - Continued

	Research Approaches	Activities: Year 1	Year 2	Year 3	Year 4	Year 5
C.3.d	Develop early larval survey (ELS).	Develop concepts for use of ELS for evaluating efficacy of low-density treatments (mating disruption); limited field work. REW	Refine year 1 results in larger field studies.	Develop concepts for using ELS to determine efficacy of high-density treatments (NPV, B.t.).	Refine and validate year 3 results in larger field studies.	Integrate early larval survey with behavioral and epidemiological studies (sick larvae may behave differently from healthy ones, influencing the survey).

RESEARCH AREA D

Modeling and Systems

RESEARCH AREA D - Modeling and Systems

	Research Approaches	Activities Year 1	Year 2	Year 3	Year 4	Year 5
D.1.	Modeling and Systems					
D.1.a	Use the geographic information system (GIS) ArcInfo to develop methods for host resource inventory.	Obtain and compile available geographic data from Montgomery and Prince Georges Counties, MD on GIS. KWT	Digitize forest stand boundary data from maps, aerial photogrammetry, and ground surveys and add to database.	Add gypsy moth density and defoliation data to database.	Partition wooded parcels into stands that vary in susceptibility to gypsy moth.	Add street trees and individual trees to database.
D.1.b	Develop computer software to manage data, facilitate interactive data analysis, and assist decision-making relative to the use of NPV in managing gypsy moth.	Determine aspects of management program requiring computer assistance. KWT	Design computer system and user interface.	Incorporate results of research on gypsy moth management using NPV into expert decision-making system.	Validate components of system by comparisons with real-world decisions.	Provide software to gypsy moth managers and attempt further validation.
D.1.c	Develop nonforest host resource monitoring system.	Develop conceptual model for priority-setting for gypsy moth monitoring. KWT	Validate conceptual model through examinations of existing management programs and focus groups with managers and homeowners.	Develop methods to use GIS to establish sampling priorities in nonforest areas.	Develop software and user interface for computerized priority-setting based on host resource inventory.	Validate GIS-based priorities by comparison with conventional priority-setting approaches.
D.1.d	Develop decision support guides (DSG).	Refine DSG for B.t. use in parks. REW	Develop DSG for B.t. use in communities.	Refine DSG for B.t. use in communities; develop DSG for NPV use in parks.	Refine DSG for NPV use in parks; develop DSG for NPV use in communities.	Refine DSG for NPV use in communities.

RESEARCH AREA D - Continued

		Activities				
		Year 1	Year 2	Year 3	Year 4	Year 5
D.1.e	Estimate costs of components of urban parks management system.	Estimate costs of B.t. & Dimilin use, survey, & oversight. RLR	Validate costs estimated in year 1.	Integrate NPV option into cost estimates.	Develop costs for various survey options.	Design optimum urban parks program and estimate and refine cost estimates of all components.
D.1.f	Develop and validate models for estimating efficacy/cost relationships for aerially applied controls.	Develop conceptual model. RLR	Incorporate incremental efficacy/cost for B.t.	Incorporate incremental efficacy cost estimates for Dimilin.	Incorporate incremental efficacy cost estimates for NPV.	Incorporate efficacy/cost comparisons for B.t., Dimilin, and NPV into a single model.
D.1.g	Develop cost-effective gypsy moth egg mass monitoring programs for suburban parks, residential areas, and individual trees.	Develop egg mass inventory methodology for parks, residential areas, and individual trees; conduct egg mass inventories. KWT	Conduct computer simulations of various potential sampling methods; conduct additional studies of sampling costs and sources and effects of bias and error.	Conduct analyses of cost-effectiveness of various potential sampling methods in parks, residential areas, and individual trees.	Conduct studies of feasibility of potential sampling methods, including studies involving park managers, homeowners, and arborists.	Validate sampling program by comparing decisions based on new sampling protocol with those using traditional methods in parks, residential areas, and on individual trees.
D.1.h	Develop a predictive population model containing the components needed to develop computerized decision support systems for parks and homesites.	Develop conceptual framework for model. RLR	Develop egg mortality sub-model.	Develop larval and pupal mortality sub-model.	Develop defoliation sub-model.	Integrate sub-models into predictive population model.

VI. APPENDICES - Management Units (Accomplishments, Objectives, Significance, Constraints, Cooperators, Research Needs)

APPENDIX A. Insect Biocontrol Laboratory

ARS GYPSY MOTH PROGRAM

Name: J. L. Vaughn

Laboratory: Insect Biocontrol

Address: Bldg. 011A, Room 214

BARC-W, Beltsville, MD

CRIS #: 1275-22000-008-00D

Telephone No.: 301-504-6328

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A. Research Accomplishments (up to 5) in Last Five Years:

1. Culture of gypsy moth cells in medium with commercial supplements in place of fetal bovine serum.

2. Culture of gypsy moth cells in medium using peptone-based media without any serum or serum replacement supplements.

B. Research Objectives for Next Five Years (brief description):

Develop medium for multiplication of NPV without serum or serum replacements.

Develop protocols for sub-scale suspension culture of gypsy moth cells and virus replication.

1. Purpose:

To reduce cost of *in vitro* production.

2. Significance:

Production of gypsy NPV *in vitro* at competitive prices is required.

3. Constraints:

Tendency of virus to multiply only as the extra cellular virion and not as polyhedral form. Lack of understanding of effects of nutrition on the replication process.

C. Current and Future Cooperators (ARS and Others): No entry.

D. Potential Uses of Research Findings:

Other scientists
Industries producing viruses for pest management.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels): No entry.

ARS GYPSY MOTH PROGRAM

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A. Research Accomplishments (up to 5) in Last Five Years:

1. Work on UV screens has identified groups of chemicals as effective screens and has provided information of mechanisms of UV inactivation and protection. Stilbene brighteners will be used as UV screens for the gypsy moth NPV (LdMNPV).

2. Certain chemicals were shown to enhance viral activity, by acting as stressors (boric acid) or by acting on host midgut (chitinase).

3. Certain optical brighteners were shown to enhance insect viruses and to increase host range.

4. In vivo selection has been successful and more virulent LdMNPV biotypes were obtained.

Research areas (3) and (4) have resulted in successful patents and licensing by Industry.

B. Research Objectives for Next Five Years (brief description):

The broad objective is to develop a fundamental understanding of factors influencing (1) pathogenicity or virulence of the NPV; (2) host susceptibility to the NPV; and (3) UV stability of the NPV.

1. Purpose: The ultimate purpose of the research is to make better use of the virus as a microbial control agent, by understanding the host-pathogen-environment interrelationships.

2. Significance: Studies on selection have led to a more "potent" virus. Studies on virus enhancers have led to the discovery of a group of chemicals (i.e., stilbene optical brighteners), as adjuvants for insect pathogenic viruses.

3. Constraints. Not enough personnel.

C. Current and Future Cooperators (ARS and Others):

ARS: E. Dougherty, D. Lynn, J. Adams, W. Nickle, R. Webb (BARC); J. Hamm (Tifton, GA); C. Ignoffo (Columbia, MO); M. McGuire (Peoria, IL).

FS: J. Podgwaite (Hamden, CT); J. Robertson (Berkeley, CA). Cooperators associated with the S-240 working group on microbial control include G. Nordin (U. Kentucky), L. Falcon (U. Calif., Berkeley), S. Young (U. Arkansas), W. Moar (Auburn U.), D. Roberts (BTI, Ithaca, NY).

Industry: B. Black (American Cyanamid Co.); M. Ticehurst (Gypsy Moth Pest Management Co.).

D. Potential Uses of Research Findings:

The selected LdMNPV is an integral component of an *in vitro* virus production system, using a fat body cell line originated by D. Lynn. This virus-cell system represents the first *in vitro* system to be produced and used commercially as a microbial control agent for insect population control. Information on the selection process is very important for the successful use of the virus. The use of stilbene optical brighteners as UV protectants and activity enhancers will not only (1) be responsible for the success of LdMNPV as a microbial control agent, (2) will greatly reduce virus production costs, and (3) will eventually be used with other insect viruses as UV protectants and/or enhancers. The successful use of these materials will accelerate the use of entomopathogenic viruses to reduce insect host populations.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

ARS has very limited personnel available to conduct research on gypsy moth NPV. It would be very advantageous to form interagency research teams (FS and ARS) to work together, to solve research problems. We really need a joint cooperative (not competitive) research effort, probably located within a single research facility, to accomplish the program needs.

ARS GYPSY MOTH PROGRAM

Name: Edward M. Dougherty

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A. Research Accomplishments (up to 5) in Last Five Years:

1. ADODR on tech transfer agreement between ARS and American Cyanamid for gypsy moth nuclear polyhedrosis virus (LdNPV).

2. Investigated permissive and nonpermissive *in vitro* systems with respect to LdNPV replication.

3. Made initial report (Guzo-Post Doc) of virus derived factor which inhibits protein synthesis in *L. dispar* cells.

4. Described partial activity of fluorescent brightener (FB) virus enhancement in the insect.

B. Research Objectives for Next Five Years (brief description):

Work on site of action of fluorescent brighteners with LdNPV *in vivo*.

Develop cell and insect transformation systems from virally derived vectors.

Identify barriers to host range of insect viruses (NPV-especially in midgut).

Utilize insect virus components for novel biocontrol agents.

1. Purpose:

   a. The utility of knowing how fluorescent brighteners work will allow further utilization of these compounds.

   b. More stable transformed insects could be made to express genes necessary for control and ecological studies.

   c. The host range of the NPV's at present remains undefined.

d. Transgenic plants with toxic specific moieties can control insects cheaply.

2. Significance:

a. Wider use of these compounds with viral systems will initiate a whole new biocontrol arena-insect viruses.

b. Sexing and sterilizing insects can become cheaper, more efficient and more effective for biocontrol purposes.

c. Knowledge of host range restriction will allow designs to widen host ranges of insect viruses.

d. Insect biocontrol via the plant itself (transgenic plants) is a thrust of the future.

3. Constraints:

Constraints are currently financial in nature. CRIS funds are at @ 160K/yr. A few pieces of equipment (microinjection apparatus, protein purification systems) and a small amount of training (80K for both) are needed. In addition, funds to supplement post docs are critically needed. If the goal of 250,000/SY were met there would be no problems.

C. Current and Future Cooperators (ARS and Others):

Ronald Weiner - U. of MD, Dept. of Microbiology
Martin Shapiro - ARS, IBL, BARC
Dwight Lynn - ARS, IBL, BARC
K. Shields - F.S., Hamden, CT
G. Bernon - APHIS, Otis, MA
T. Czapala - Pioneer Hybrid Inc., Johnson City, IA

D. Potential Uses of Research Findings:

Industry - Mainly American Cyanamid Inc. - Development and commercialization of L. dispar NPV. FMC, Sandoz and ICN also utilize virus technology. Transformed cell lines will be used by the biomedical industry.

APHIS - Transformed insects can be genetically sexed and/or sterilized with proper genetic manipulation. Also, transformed insects can be tagged and followed in the environment.

Seed Industry - Transgenic plants will be utilized by the seed industry.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Gypsy moth research is an orphan within ARS. Several important areas of gypsy moth research, especially microbials, can be commercialized yet commitments lag for developing these programs and counting them as a true program.

ARS GYPSY MOTH PROGRAM

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CRIS #: 1275-24000-043-00D

Telephone No.: 301-504-6332

FAX #: 301-504-5104

A. Research Accomplishments (up to 5) in Last Five Years:

1. Identified a new pleomorphic microorganism causing problems in many insect mass rearings.

2. The pathogen is a rickettsia-like organism (RLO). The life cycle has been determined.

3. The pathogens occurring in gypsy moth larvae in Maryland experimental field plots were identified.

4. The DNA of the RLO, a very fragile organism, was isolated.

B. Research Objectives for Next Five Years (brief description):

Study the effect of the fluorescent brightener on LdMNPV in the gut of the gypsy moth using light and electron microscope immunocytochemical techniques.

1. Purpose:

To study mode of action of fluorescent brightener and to better understand the invasion and replication of NPVs in vivo.

2. Significance:

Methods for microbial control of insect pests can be improved as further details of the process of invasion and replication are elucidated.

3. Constraints: No Entry.

C. Current and Future Cooperators (ARS and Others):

Dr. Carol Sheppard
Dr. Ed Dougherty
Dr. Martin Shapiro

D. Potential Uses of Research Findings:

Improve activity of LdMNPV and possible other insect viruse.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels): No entry.

ARS GYPSY MOTH PROGRAM

Name: Dwight E. Lynn

Laboratory: Insect Biocontrol

Address: Bldg. 011A, Rm. 214

BARC-West, Beltsville, MD

CRIS #: 1275-22000-008-00D

Telephone No.: 301-504-6328

FAX #: 301-504-5104

A. Research Accomplishments (up to 5) in Last Five Years:

1. Development of new gypsy moth cell lines including a fat body derived line with higher yields of gypsy moth nuclear polyhedrosis virus (NPV).

2. Selection and screening of clonal isolates of the Abington strain of gypsy moth NPV.

3. Development of a process for infecting cell cultures with the occluded form of NPV.

B. Research Objectives for Next Five Years (Brief Description):

Research on improving the infection of cell cultures with the occluded form of the virus will be completed with emphasis on long term passage of virus in cell culture.

Other research on gypsy moth will be phased out over the next year or so.

1. Purpose:

   a. To maintain high-yielding infection of cell cultures with NPV over long term.

2. Significance:

   This is necessary for commercial production of NPV in large-scale cell cultures.

3. Constraints: No entry.

C. Current and Future Cooperators (ARS and Others):

In Insect Biocontrol Lab: E. M. Dougherty
M. Shapiro

Plus several researchers at American Cyanamid (San Leandro).

D. Potential Uses of Research Findings:

Large-scale production of NPV for biopesticide use.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

No entry.

ARS GYPSY MOTH PROGRAM

Name: Richard L. Ridgway

Laboratory: Insect Biocontrol

Address: Bldg. 402, Room 107

BARC-E, Beltsville, MD

CRIS #: 1275-24000-025-00D

Telephone No.: 301-504-9028

FAX #: 301-504-9077

A. Research Accomplishments (up to 5) in Last Five Years:

1. High summer temperatures inhibit gypsy moth pheromone production. At moderate temperatures, both male flight and female pheromone production increased synchronously throughout the day. However, at high (33°C) temperatures, pheromone production was inhibited. These results indicate that the spread of the gypsy moth to southern states may be hindered by impaired mating communication.

2. Pheromone trap-based monitoring system. The use of pheromone traps to provide information about gypsy moth population density was investigated using 1- or 500-µg (standard) lures. Functional relationships between trap catch and subsequent egg mass density for traps with low and high dose lures were developed. These results suggest that traps could be used to evaluate the efficacy of spray blocks and possibly indicate whether a spray block needs to be resampled for egg masses in successive years.

3. Gypsy moth management in suburban parks. A specialized gypsy moth program was designed for use in suburban parks and other public lands. A decision guide was developed based on number and size of egg masses, host susceptibility, and previous defoliation. One or two applications of *Bacillus thuringiensis* (*B.t.*) or one application of diflubenzuron (Dimilin™) were applied with the goal of preventing defoliation from exceeding 30%. Over the course of four years, program goals were met on 96.7% of the 20,385 acres included in the study.

4. Private control costs in residential areas. Households in Maryland and Pennsylvania were surveyed to estimate their valuations for gypsy moth control programs. Over 20% of the sample of 436 households reported dollar expenditures for private control measures, with 14% using spray methods, 53% spending $25 or less, and 7% spending more than $250.

B. Research Objectives for Next Five Years (brief description):

Develop an analytical understanding of gypsy moth larval and adult behavior, with emphasis on interactions with management tactics and survey technology; develop/refine methods for quantifying gypsy moth egg mass, larval, and pupal population densities, and use these methods to develop/refine operationally feasible and cost-effective methods for quantifying natural mortality factors and field efficacy of new management technologies.

Develop/refine microbial suppression agents against gypsy moth appropriate for use in urban/suburban areas and areas of environmental sensitivity; quantitatively evaluate efficacy of selected NPV doses and formulations for control of gypsy moths in parks using methodology

previously developed for use of *B.t.*; develop/refine technology suitable for gypsy moth suppression by homeowners and/or communities (*i.e.*, ground applications, barrier bands).

Develop improved survey methods, including egg mass sampling and pheromone traps, appropriate for use in low and high density gypsy moth populations; develop computerized decision aids, including knowledge-based and resource inventory software, to assist the implementation of gypsy moth management technologies.

Integrate information obtained on natural and induced gypsy moth mortality with information on gypsy moth population dynamics and distribution into a decision guide to optimize gypsy moth control; conduct selected economic assessments of tactics and model systems through cooperative agreements as the needs arise and funds permit.

1. Purpose:

   The objectives are designed to reflect a project designed to develop and integrate the components of integrated pest management programs (IPM): biology, suppression tactics, decision support, and systems. The IPM programs involved are to be specifically designed for non-forest areas such as parks, communities, and individual home sites.

2. Significance:

   The development of improved IPM programs for the various specialized non-forest areas will reduce loss of valuable trees and reduce the nuisance effects associated with the gypsy moth.

3. Constraints:

   Laboratory and green house facilities are not adequate to obtain maximum benefit from available resources.

C. Current and Future Cooperators (ARS and Others):

Robert Tichenor, Maryland Department of Agriculture, Annapolis, MD
Ken Farminer, AgriSense, Fresno, CA
Susan Burkhart, American Cyanamid Co., Princeton, NJ
Shelby Fleischer, Pennsylvania State University, University Park, PA
Vernon Illum, CCT Corporation, Shawnee, KS

D. Potential Uses of Research Findings:

There is an unmet need for environmentally compatible tactics and systems for use in urban environments that provide the assured foliage protection required for high-value urban shade and landscape trees. The proposed research should mitigate current problems through the development of systems featuring improved sampling protocols, control tactics, and evaluation criteria specifically design for the particular needs of the urban environment.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

Although substantial resources are devoted to gypsy moth research by ARS, an overall strategy for integrating all research activities is not evident. More interactive planning, in which the relationship between fundamental and applied research is examined, and a specific approach to integration into the total research and development continuum is needed. This approach should result in an improved understanding by the scientists involved of the specific problems to be addressed and the best means of interaction to solve those problems.

ARS GYPSY MOTH PROGRAM

Name: Ralph E. Webb

Laboratory: Insect Biocontrol

Address: Bldg. 402, Room 201

BARC-E, Beltsville, MD

CRIS #: 1275-24000-025-00D

Telephone No.: 301-504-8262

FAX #: 301-504-9077

A. Research Accomplishments (up to 5) in Last Five Years:

1. Improved strains and production of gypsy moth virus. Field evaluations indicated that an experimental strain (Abington) of the gypsy moth nuclear polyhedrosis virus (NPV) killed gypsy moth larvae faster than the strain (Gypchek) currently approved for practical use.

2. Potentiation of the gypsy moth virus. The field efficacy of optical brighteners (Phorwite AR at 1.0%, Blankophor BBH at 0.5% and 0.05%) plus Gypchek was evaluated against a standard formulation of Gypchek plus Orzan (a sunscreen) in small (0.02 ha) forest plots. Both optical brighteners clearly potentiated the virus, resulting in increased larval mortality and speed of kill, for both the natural virus in the field and for applied virus.

3. Vegetable and mineral oils effective ovicides. In cooperation with USDA-APHIS, fall and spring applications of oils, soaps, or pesticides were evaluated as gypsy moth ovicides in New England and in Maryland. Oils (soy oil and Sun Superior oil) caused high mortality (> 99%) when applied as 3:1 water:oil emulsions.

4. Barrier bands for gypsy moth suppression. The systematic application of sticky barrier bands to over 300 trees in a community where gypsy moth populations were threatening prevented noticeable defoliation. An experimental comparison of groups of banded trees with unbanded trees indicated that banding of trees reduced defoliation.

5. Impact of control tactics on natural enemies - Selected environmental impacts of aerially-applied gypsy moth suppression tactics — Gypchek, *Bacillus thuringiensis* (*B.t.*), and diflubenzuron — were determined. Two important components of the gypsy moth natural enemy complex (natural NPV and the parasitoid *Cotesia melanoscela*) varied in their compatibility with these tactics.

B. Research Objectives for Next Five Years (brief description):

Develop an analytical understanding of gypsy moth larval and adult behavior, with emphasis on interactions with management tactics and survey technology.

Develop/refine microbial suppression agents against gypsy moth appropriate for use in urban/suburban areas and areas of environmental sensitivity. Quantitatively evaluate the efficacy of selected NPV doses and formulations for control of gypsy moths in parks using methodology previously developed for use with *B.t.* Develop/refine technology suitable for gypsy moth suppression by homeowners and/or communities (*i.e.*, ground applications, barrier bands).

Integrate information obtained on natural and induced gypsy moth mortality with information on gypsy moth population dynamics and distribution into a decision guide to optimize gypsy moth control.

1. Purpose:

   a. To provide a behavioral underpinning for development of new/improved control tactics and integrated systems.

   b. To provide cost-effective environmentally-friendly control tactics especially designed to protect high-value trees in non-forest situations.

   c. To integrate all available information into systematic programs for the areawide suppression of gypsy moths in non-forest situations.

2. Significance:

   a. Improved use of control tactics/strategies for gypsy moth control.

   b. Improved control tactics/strategies for gypsy moth control in non-forest situations.

   c. Improved systems for gypsy moth control in non-forest situations.

3. Constraints:

   Reduced Agency support/understanding of the importance/constraints of field research to provide real-world solution to real-world problems and to provide real-world evaluation of the fruits of Agency laboratory discoveries.

C. Current and Future Cooperators (ARS and Others):

American Cyanamid Co. (Contact: Susan Burkart)
Norman Dill, Del. St College, Dover, DE
Joseph Maddox, INHS, Champaign, IL
Winford McLane, APHIS, Otis ANGB, MA
Michael McManus, USFS, Hamden, CT
Karl Mierzejewski, Penn St U, University Park, PA
John Podgwaite, USFS, Hamden, CT
Richard Reardon, USFS, Morgantown, WV
Robert Tichenor, Md Dept Agr, Annapolis, MD

D. Potential Uses of Research Findings:

Development of gypsy moth tactics and strategies that provide cost-effective environmentally friendly control tactics especially designed to protect high-value trees in non-forest situations, with all available information incorporated into systematic programs for the areawide suppression of gypsy moths in non-forest situations.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

The most important program that USDA could undertake would be the introduction of important components of the gypsy moth natural enemy complex, specifically the cytoplasmic polyhedrosis virus (CPV) and a complex of microsporidia that thrive in Eurasian gypsy moth populations but are absent from North America.

ARS GYPSY MOTH PROGRAM

Name: Kevin W. Thorpe

Laboratory: Insect Biocontrol

Address: Bldg. 402, Room 204

BARC-E, Beltsville, MD

CRIS #: 1275-24000-025-00D

Telephone No.: 301-504-5139

FAX #: 301-504-9077

A. Research Accomplishments (up to 5) in Last Five Years:

1. Assessment of gypsy moth mortality by a parasitoid. Successful parasitism by *Cotesia melanoscela* was greatest when larvae were from 3 to 6 days old. Significant levels of non-emergence mortality occurred only when larvae were less than 3 or more than 6 days old.

2. Gypsy moth egg mass sampling on urban homesites. Based on cost-effectiveness criteria, a binomial (presence/absence) sampling approach may provide a preferred sampling method for use in residential areas.

3. Quantification of larval populations. Frass collection techniques were used to quantify absolute gypsy moth larval numbers in tree canopies, and to measure larval movement along the boles of trees and through the canopy. There was no evidence of net lateral movement through the canopy, and net movement of larvae into the canopy along the bole ranged from 5 to 35% of the total number in the canopy.

4. Computerized decision-support system for gypsy moth management. A computerized decision support system was developed to address specific data processing and decision support needs for gypsy moth management in suburban parks.

B. Research Objectives for Next Five Years (brief description):

Conduct research on gypsy moth behavior and biology, with emphasis on population dynamics, ecology, natural mortality factors, and larval feeding behavior.

Develop improved and appropriate control tactics for non-forest environments, including barrier bands and biological agents.

Develop improved and cost-effective methods for sampling populations, and determine relationships between gypsy moth density and damage, including defoliation and nuisance.

Develop integrated systems, with emphasis on computerized decision-support tools and geographic information systems.

1. Purpose:

Better understanding of gypsy moth biology and population dynamics will result in more successful implementation of management tactics. The use of more cost-effective sampling methods will increase the effectiveness of current gypsy moth management programs.

Improved methods of quantifying larval mortality will result in greater sensitivity in determining the effectiveness of control tactics.

2. Significance:

More sensitive methods for assessing larval mortality would reduce the impact of natural population collapse on field trials of control tactic efficacy and would increase the probability of obtaining useful research results. Improvements in the cost-effectiveness of sampling methods would increase the value of the information obtained from survey activities and could allow more resources to be used for suppression activities. Quantification of gypsy moth density/damage relationships would facilitate the development of suppression thresholds that are based on data from relevant management environments, such as residential areas and suburban parks.

3. Constraints:

Meaningful research on gypsy moth populations requires large wooded field plots that meet stringent population density and quality criteria. Locating such plots within a reasonable travelling distance from Beltsville is becoming increasingly difficult. Available laboratory and green house facilities are not adequate.

C. Current and Future Cooperators (ARS and Others):

Robert Tichenor, Maryland Department of Agriculture, Annapolis, MD
Hobson Calhoun, Prince Georges County, Landover, MD
Michael Raupp, University of Maryland, College Park, MD
Barbara Leonhardt, Insect Chemical Ecology Laboratory, ARS, USDA, Beltsville, MD
Karl Mierzejewski, Pennsylvania State University, University Park, PA

D. Potential Uses of Research Findings:

Development of gypsy moth sampling protocols utilizing improved sampling methods for use by government agencies, industry, and homeowners. Development of gypsy moth management programs for specific management environments, including suburban parks, residential areas, and other non-forest situations. Development of specific tactics that are effective and that meet the needs of specific management programs and user requirements.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

The development and commercialization of appropriate management tactics for gypsy moths, especially in non-forest and wilderness areas, are not currently being met by the commercial sector alone because of high costs of development, regulatory constraints, and relatively small market sizes for commercial products. Public agency involvement will be required for the research and development of new technology for gypsy moth management, especially highly specific biological agents and non-product technology such as sampling methods and decision-support systems.

ARS GYPSY MOTH PROGRAM

Name: Dr. Robert F. W. Schroder

Laboratory: Insect Biocontrol Laboratory

Address: Bldg. 406, Room 01, BARC-East

Beltsville, MD 20705

CRIS #: 1275-22000-091-00D

Telephone No.: 301-504-8369

FAX #: 301-504-8067

A. Research Accomplishments (up to 5) in Last Five Years:

Conducted study of 20 woodloots in western Maryland and southwestern Pennsylvania for forest insect-parasite complexes prior to and after arrival of the gypsy moth (GM). Determined alternate host for targeted introduced GM parasites. The lymantriid, Dasychira meridionalis is the most promising alternate host studied. This moth overwinters as a larvae and its preferred host is white oak. Distribution is from the MD - PA border into the SE U.S.

B. Research Objectives for Next Five Years (brief description):

a. To determine the host-parasite interactions of GM parasites with D. meridionalis in SE U.S.

b. Determine suitability of D. meridionalis as an alternate host for introduced GM parasites.

1. To collect D. meridionalis in adequate numbers to establish a laboratory colony. Expose D. meridionalis to select GM parasites under various laboratory conditions.

2. This research will help determine if D. meridionalis can serve as a reservoir for overwintering GM parasites. The absence of alternate hosts for introduced GM parasites has been a major problem in the GM biocontrol program in the U.S.

3. Adverse climatic conditions in the past several years and other unknown factors have dramatically reduced the number of D. meridionalis collected. As a matter of fact, the only species collected in 1991 was D. basiflava.

C. Current and Future Cooperators (ARS and Others):

Gary Bernon, APHIS, OTIS Methods and Development Center, MA
Richard Ridgway, IBL, Beltsville, MD
Paul Schaefer, USDA, BIRL, Newark, DE

D. Potential Uses of Research Findings:

Provide new information on potential alternate hosts for introduced GM parasites that until now have not had suitable overwintering hosts.

E. Thoughts on Research Needs (not being addressed in other Agencies or at State levels):

If we establish a DM colony in the laboratory and have success with GM parasites, then we will need more funds and technical support. At the present, less than 20% of our efforts is towards the GM.

ARS GYPSY MOTH PROGRAM

Name: Fredrick I. Proshold

Laboratory: Insect Biocontrol Laboratory

Address: USDA, APHIS, Bldg. 1398

Otis ANGB, MA 02542

CRIS #: 1275-22000-066-00D

Telephone No.: Comm. 508-563-9303

FTS 828-9355/54

A. Research Accomplishments (up to 5) in Last Five Years:

1. Dietary moisture can range from 68 to 80% without reducing survival or development in laboratory-reared gypsy moths. More variation in survival and development was observed in recently colonized strains than one that had been colonized for 35 generations.

2. In laboratory studies, female gypsy moth can mate more than once. If they receive only apyrene sperm from the first male, they can receive eupyrene sperm from a second male even though held for 24 hours before remating.

3. F_1 males from gypsy moths crossed with irradiated males do not transfer eupyrene sperm as frequently as nonirradiated males.

B. Research Objectives for Next Five Years (brief description):

Describe the reproductive biology of the Asian gypsy moth (AGM) and hybrids of the AGM X European strain.

Carry hybrid studies from AGM X European gypsy moth through the third generation and compare growth, survival, fertility and fecundity of all crosses.

Compare the reproductive biology of laboratory and wild gypsy moth strains and determine factors contributing to APS or other problems in mass rearing.

Determine whether low level, isolated populations of gypsy moths can be controlled by F_1 sterility.

1. Purpose:

Determine the compatibility of the AGM and American strain as well as the hybrids and backcross progeny for three generations.

Improve the mass rearing capabilities of gypsy moth by determining problems that may arise in reproduction because of rearing procedures.

Define in detail all aspects of mating fitness of F_1 insects in the laboratory and interaction between F_1 and native insects in the field so that the potential of this technique can be critically assessed.

2. Significance:

Three infestations of the Asian gypsy moth have been discovered in North America. Because females of this strain are strong flyers, the AGM has aroused much concern. It is estimated that the threat of this insect may be in the billions of dollars. Because of regulation involving the AGM and proposed survey and control activities, it is important that the USDA conduct specific studies to describe this strain's biology, behavior, and compatibility with the American strain which originated from Europe. Early work by Goldschmidt showed that hybrids from some interracial crosses developed into sterile intersexes. Recent work by Clark has indicated that these are not intersexes but that the sex ratio distortion is caused by death of the heterogametic sex because of the "Haldane" factor. Thus, compatibility between these two strains needs to be addressed to more thoroughly understand the threat of increasing the genetic variability of this insect through introgression.

As long as the F_1 sterility and in vivo production of virus remain viable control strategies, mass rearing technology will be important. Although population suppression has been demonstrated by releasing irradiated males, the labor involved in rearing gypsy moths in sufficient numbers for release is impractical. Consequently, technology to distribute eggs from females crossed with irradiated males in the laboratory has been developed. Insects from these eggs are sterile, but whether they can suppress native populations remains inconclusive. In laboratory studies, F_1 males do not transfer eupyrene sperm as frequently as nonirradiated males. If females crossed with F_1 males are monogamous, then suppression depends upon synchronous development and eclosion of released insects with native insects and upon the ability of released insects to secure mates. But if females will mate more than once in nature as they do in small laboratory cages, then suppression also depends upon competition of sperm transferred by the released male. There is little published information on the mating fitness of released F_1 insects and their interaction with native insects.

3. Constraints:

The Asian gypsy moth must be kept in quarantine. There may be seasonal restrictions placed upon certain studies as a precaution against accidental escape.

APPENDIX B. Insect Chemical Ecology Laboratory

ARS GYPSY MOTH PROGRAM

Name: Barbara A. Leonhardt

Laboratory: ICEL/PSI

Address: Building 011A

Rm 165A, BARC-West

Beltsville, Maryland 20705

CRIS #: 1275-24000-089-00D

Telephone No: 301-504-6394

FAX #: 301-540-6580

A. Research Accomplishments (up to 5) in Last Five Years:

Developed a low-dose pheromone dispenser now being evaluated for population measurement.
Invented a new PVC/twine pheromone dispenser as an alternate for the laminate in detection traps.
Led the development and evaluation of a new commercial microbead formulation of racemic disparlure for mating disruption.

B. Research Objectives for Next Five Years (brief description):

Complete the development and implementation of the new PVC/twine pheromone dispenser for detection.
Determine the relationship between dose and mating disruption efficacy for the microbead formulation of disparlure.
Design and test alternate formulations for use in mating disruption.
Identify pheromone of Asian gypsy moth
Compare cuticular waxes of Asian and North American gypsy moths to look for differences which could be useful for identification.

1. Purpose:
   Develop more effective tools for use in detection and control.
   Develop the best pheromone lure for detection of Asian gypsy moth.
   Find chemical cues to distinguish Asian and North American gypsy moths.

2. Significance:
   The new PVC/twine dispenser will be far less expensive and longer lasting than the currently used laminate.
   The new microbead formulation can be sprayed with conventional aircraft spray systems and probably is effective at much lower application rates than the currently used laminate flake formulation.
   New methods are needed to detect and distinguish Asian gypsy moth.

3. Constraints:
   Large field tests are expensive and different to run. Close cooperation with FS, APHIS and commercial firms is required. Only one SY in ARS is working development of on pheromone formulations for gypsy moth management.

C. Current and Future Cooperators (ARS and Others):

FS
APHIS
AgriSense
Farma Tech International
State Depts. of Agriculture

D. Potential Uses of Research Findings:

In detection traps for North American and Asian gypsy moth males as part of APHIS programs.
For control of low - level populations by mating disruption as part of pest management programs.
For distinction of Asian and North American gypsy moths

E. Thoughts on research Needs (not being addressed in other agencies or at state level):

None

APPENDIX C. Insect Neurobiology and Hormone Laboratory

ARS GYPSY MOTH PROGRAM

Name: Thomas J. Kelly

Laboratory: Insect Neurobiology & Hormone Laboratory

Address: USDA-ARS-INHL
Bldg. 306, Rm. 322, BARC-East
10300 Baltimore Blvd.
Beltsville, MD 20705-2350

CRIS #: 1275-22000-069-00D (50%)
1275-22000-070-00D (50%)

Telephone No.: 301-504-8787 (FTS 964-8787)

FAX #: 301-504-8190

A. Research Accomplishments (up to 5) in Last Five Years:

In cooperation with other INHL scientists:
1) Developed in vitro and in vivo bioassays for gypsy moth prothoracicotropic hormone (PTTH)
2) Characterized gypsy moth vitellogenins and vitellin and demonstrated a novel suppression mechanism by juvenile hormone.
3) Demonstrated ecdysone and 3-dehydroecdysone production by gypsy moth prothoracic glands and a novel egg ecdysone ketoreductase.
4) Demonstrated a novel form of PTTH in gypsy moth eggs.
5) Obtained a partial nucleotide sequence for the gypsy moth PTTH gene.

B. Research Objectives for Next Five Years (brief description):

(CRIS #1275-22000-069-00D) Identify physiological processes by which the gypsy moth central nervous system controls and regulates development, reproduction and diapause, and develop bioassays for these factors.
(CRIS #1275-22000-070-00D) Identify, isolate, and structurally characterize neurohormones and related peptidergic factors from the gypsy moth.

1. Purpose: To develop knowledge of hormonal and neurohormonal regulation of gypsy moth growth, development, reproductive and behavioral processes as a basis for discovering new control principles.

2. Significance: Many vital functions are controlled by hormones and neurohormones and many of these regulatory factors, especially the neurohormones, have not been characterized in the gypsy moth.

3. Constraints: Inadequate knowledge of these factors and their regulatory mechanisms hampers the development of new control methods.

C. Current and Future Cooperators (ARS and Others):

Dr. Bruce Black, American Cyanamid Company, Princeton, New Jersey
Dr. Lois Miller, University of Georgia, Athens
Dr. David Borst, Illinois State University, Normal
INHL Scientists

D. Potential Uses of Research Findings:

1) The basic knowledge acquired on the structure and regulation of gypsy moth yolk proteins, neuropeptides and their genes provides necessary information for development of new control methods based on disruption of vital functions.

2) Useful quantities of gypsy moth neurohormones such as PTTH, invaluable in future research on receptor isolation, processing, etc., will be provided by vector expression of the purified genes.

E. Thoughts on Research Needs (not being addressed in other agencies or at state level):

Research on fundamental biology of the gypsy moth needs a significant increase in funding to address many other exploitable areas of hormonal and neurohormonal regulation of gypsy moth development, reproduction and behavior. These problems are currently being addressed by only three major U.S. groups: one in our laboratory, one at the University of Massachusetts, Amherst and one at the USDA, Forest Service lab in Delaware, Ohio. For an overview of current research in this area see Kelly, T. J., Fundamental biology of the gypsy moth: a summary of current research results. In: Proceedings of the USDA Interagency Gypsy Moth Research Review 1991, M. J. Twery and K. W. Gottschalk, eds., USDA-Forest Service, Northeastern Forest Experiment Station, in press.

ARS GYPSY MOTH PROGRAM

Name: Dale B. Gelman

Laboratory: INHL

Address: Bldg. 306, Rm. 317

BARC-East

Beltsville, MD 20705

CRIS #: 1 275-24000-070-000

Telephone No.: 301-504-8909

FAX #: 301-504-8190

A. Research Accomplishments (up to 5) in Last Five Years:

Discovered the presence of an ecdysiotropin (stimulates the production of an ecdysteroid which is a precursor to the molting hormone) in the hindguts of the gypsy moth and the European corn borer.

B. Research Objectives for Next Five Years (brief description):

Isolate and purify the ecdysiotropic peptide. Sequence the ecdysiotropin. Determine the physiological role(s) of the peptide. Produce antibodies to the peptide to facilitate its detection so that its distribution in the insect as well as its site of production can be located.

1. **Purpose:** To isolate, characterize and determine the mode of action of a newly discovered ecdysiotropic peptide from the insect hindgut.

2. **Significance:** To utilize this knowledge to design and synthesize new agents that will interfere with insect molting and metamorphosis, and thus act to control insect pests.

3. **Constraints:** This peptide is very potent but is present in miniscule amounts in the insect hindgut. Active fractions are not producing a visible peak on the chromatogram. This will necessitate dissecting and processing very large quantities of hindguts. Additional student help would be useful.

C. Current and Future Cooperators (ARS and Others):

Dr. Robert Bell INHL Beltsville, MD
Dr. Renee Wagner LIL Beltsville, MD
Dr. Jan Kochansky INHL Beltsville, MD

D. Potential Uses of Research Findings:

Design and implement additional alternative environmentally-safe control strategies for managing gypsy moth populations.

E. Thoughts on Research Needs (not being addressed in other agencies or at state level): No entry.

ARS Gypsy Moth Program

Marcia J. Loeb
Insect Neurobiology and Hormone Laboratory
Bldg. 306, Rm. 319, BARC East
Beltsville, MD 20705

CRIS #: 1275-22000-070
Telephone #: 301-504-8103
Fax #: 301-504-8190

A: Research Accomplishments

Research: REPRODUCTIVE PHYSIOLOGY OF THE MALE GYPSY MOTH

1. Gypsy moth testes secrete ecdysteroids, and thus function as endocrine organs.

2. A brain neuropeptide, testis ecdysiotropin (TE), controls ecdysteroid secretion by the testes.

3. TE has been partially purified using high pressure liquid chromatography (HPLC).

4. TE action at the cellular level in the testis is mediated by second messengers of the inositol phosphate system and by calcium ion influx.

5. In the presence of ecdysteroids, testes secrete at least 9 different molecular weight ranges of growth factors. One or more of the growth factors must be present to allow partial development of the male reproductive tract *in vivo*.

B: Research Objectives for the Next 5 Years

1. Purpose: to attain purification of TE and its analogues from brain extracts. When truncated or changed structures of TE are synthesized, subsequent bioassays will indicate the active site (s) of TE. Synthetic molecular forms may be more or less active, or block the action of TE by sticking to TE receptors. TE can be synthesized to provide sufficient material for production of antibodies. Histochemical studies will determine the source and distribution of TE within the gypsy moth, and radioimmune assays can be developed to detect hemolymph and tissue titers of TE in order to understand how it functions physiologically. We will attempt to determine the structures and properties of reproductive tract growth factors and their modes of action.

2. Significance: Each male inseminates several females and is therefore vital to insect fecundity. Although gypsy moth males produce sperm as larvae, the genital tract develops during the short pupal stage. It may be possible to devise procedures to block genital tract development by interfering with the release or action of TE, and thus interfere with ecdysteroid production by testes, or production or function of reproductive growth factors. Study of growth factors adds to the understanding of insect reproductive physiology. Genital tract growth factors may also be useful in *in vitro* culture of other tissues heretofore difficult to maintain in the laboratory.

3. Constraints: The biochemistry and physiology of male insect reproduction appears to be complicated by many interacting factors. Untangling the web of cross-interactions will take time and

manpower. At present I have only one part time technician to assist me.

C. Current and Future Cooperators

1. Current: RA Bell, DB Gelman, J. Kochansky (INHL); R. Wagner, (LIL); D. Lynn, (IBL), U.S.D.A. Beltsville, MD; D. Hunt, University of VA, Charlottsville, VA.

2. Future: S. Meola, VTL, U.S.D.A. College Station, TX; J. Wright, Texas A and M University, College Station,TX.

D. Potential Uses of Research Findings

1. TE: Blocking TE active sites *in vivo* may inhibit the male reproductive development cascade and thus prevent attainment of male reproductive maturity.

2. Growth factors: Insect growth factors may be applied to other tissue culture systems to enhance growth of cell lines in which insect viruses and other insect pathogens are cultured.

E. Thoughts on Research Needs

Additional funding for full time support and postdoctoral assistance would speed and amplify the research.

ARS GYPSY MOTH PROGRAM

Name: E.P. Masler

Laboratory: Insect Neurobiology and Hormone Laboratory

Address: B-306 R-309

BARC-E

Beltsville MD 20705

CRIS #: 1275-22000-070-000

Telephone No.: 301-504-8732

FAX #: 301-504-8190

A. Research Accomplishments (up to 5) in Last Five Years:

1. Discovered and characterized embryonic and post-embryonic PTTH, identified two molecular weight classes of this ecdysiotropin and outlined this bi-molecular distribution in other lepidopterans.
2. Discovered a non-cerebral neurohemal center which functions independent of the brain to control development and metamorphosis, have partially characterized a PTTH-like peptide from this center, and have developed a bioassay for the peptide.
3. Isolated, purified and sequenced a pheromonotropic peptide (PBAN-like) from adult brains - the first neuropeptide ever sequenced from gypsy moth.
4. Discovered and characterized neuropeptide-metabolizing, membrane-bound enzyme activities in brain preparations; endopeptidase and aminopeptidase activities were described.
5. Discovered that juvenile hormone specifically suppresses vitellogenin (i.e. egg) production in the gypsy moth.

B. Research Objectives for Next Years (brief description):

We intend to isolate and characterize PTTH and the non-cerebral ecdysiotropin, conduct structure-function studies on gypsy moth PBAN, isolate the PBAN and PTTH genes and characterize specific neuropeptide metabolizing enzymes.

B. (con't)

1. Purpose: All studies are directed at understanding the production, action, and degradation of specific insect neuropeptides which are essential for reproduction and development.

2. Significance: The study will provide a comprehensive, vertical knowledge of neuropeptide systems which is necessary for, and will be applied to, the development of insect control agents through peptidomimetic design, baculovirus engineering, and other technologies.

3. Constraints: Essential for rapid progress in these cutting-edge programs are a molecular biologist and a support scientist trained in protein and enzyme manipulation.

C. Current and Future Cooperators (ARS and Others):

T. S. Adams (ARS, Fargo)
T. A. Coudron (ARS, Columbia)
D. Bolt (ARS, Beltsville)
Members of INHL
R. B. Imberski (U. of Maryland)
J. W. Gerst (N. Dakota State Univ.)
B. Black (American Cyanamid)

D. Potential Uses of Research Findings:

Research findings will be of value as 1) direct avenues to the development of experimental, novel pest control agents, and 2) as highly detailed reservoirs of information and methods relative to neuropeptide production, action and consequence in L. dispar and other pest species. This will significantly decrease the time and research expense necessary to identify neuropeptides which control key physiological events and to develop agents to interfere with these events.

E. Thoughts on Research Needs (not being addressed in other agencies or at state level): No entry.

ARS GYPSY MOTH PROGRAM

Name: Robert A. Bell

Laboratory: Insect Neurobiology and Hormone Lab

Address: Rm 214, Bldg 309, BARC-E

Beltsville, MD 20705

CRIS #: 1275-22000-069-00D

Telephone NO.: (301)-504- 8015

FAX #: (301)-504-8190

A. Research Accomplishments (up to 5) in Last Five Years:

(1) Determined respiratory activity during embryogenesis, diapause and chill-induced diapause termination in the gypsy moth.
(2) Established ecdysteroid and prothoracicotropic hormone (PTTH) titers during embryonic development and diapause.
(3) Determined the relationship between dose and time of application of a juvenile hormone analogue, Methoprene, on induction of increased larval weight, prolongation of development and occurrence of developmental arrest or formation of permanent larvae.
(4) Developed a practical method for prevention and precocious termination of diapause in gypsy eggs using KK-42, a novel imidazole terpenoid with anti-juvenile hormone properties.
(5) Demonstrated a wide range of developmental effects induced in the gypsy moth by exposure to varying doses of RH 5849, an ecdysteroid agonist, and KK-42, a novel anti-hormone.

B. Research Objectives for Next Five Years (brief description):

(1) Continue research to develop a comprehensive understanding of the hormonal and neurohormonal mechanisms involved in regulation of late embryonic diapause with particular emphasis on the role of juvenile hormone and the possible involvement of a special inhibitory factor or diapause hormone.
(2) Determine the genetic factors involved in gypsy moth diapause and associated cold-hardiness. Isolate and seek to identify genes and gene products (proteins) that are expressed and associated with diapause.
(3) Explore and develop methods for large scale manipulation of diapause and methods for stockpiling diapausing gypsy and selected parasites for biological control programs.
(4) Improve insect rearing and biocontrol technology by application of insects hormones, antihormones and their

analogues. The use of hormones would be explored for increasing *in vivo* production of microbiol pesticides such as viruses and certain parasites. Hormonal agents or anti-hormonal compounds would be tested in the laboratory and in the field for augmenting the efficacy of gypsy moth control by natural enemies.

1. Purpose:
   The overall purpose of the proposed research program is to develop fundamental understanding of the physiological (endocrine and genetic) mechanisms involved in late embryonic diapause and to apply knowledge gained to improve gypsy moth rearing and biocontrol technology.

2. Significance:
   The research is expected to yield new knowledge of the endocrinology and molecular genetics of insect diapause and embryonic development. These studies should also lead to isolation and discovery of novel naturally occurring compounds that will prove useful to development of new and safer pest control agents.

3. Constraints:
   Research is hampered by inadequate heating/cooling systems in the building, lack of proper insectary facilities and difficulty in obtaining good support personnel. Also, assistance of a molecular biologist will be essential for isolating genes and gene products associated with diapause.

4. Research Approaches: (see attached page)

C. Current and Future Cooperators (ARS and Others):
Current cooperators involved in this research are T. Kelly, D. Gelman, P. Masler and B. Thyagaraja (INHL) and A. DeMilo (ICEL), ARS,Beltsville. Future cooperators would be likely to involve a molecular biologist and expertise in juvenile hormone assay already available in INHL and expertise within the IBL at BARC, Beltsville. Potential cooperators have been identified at APHIS, Otis ANGB, MA, U of Maryland, U of Mass, Amherst and VPI, Blacksburg, Va and USFS, Hamden CT.

D. Potential Uses of Research Finding:

There are several potential uses of research results. The ability to manipulate diapause will circumvent the need to chill eggs for several months to obtain hatch thereby overcoming the need for refrigeration equipment and will permit production of several generations per year without intervention of diapause. Research can be carried out more rapidly and especially genetic studies which involve more than a single generation. The ability to stockpile diapausing eggs would increase the production capability of insects for the sterile release program. Isolation of compounds that

regulate insect diapause and development may lead to the synthesis of new pesticides that inhibit development and disrupt diapause in nature. Hormones and hormone analogues already available should be useful in improving production of natural enemies in rearing facilities and for enhancing their effectiveness in pest control by slowing growth in the host or by other means.

ARS GYPSY MOTH PROGRAM

Name: Ashok K. Raina

Laboratory: Insect Neurobiology and Hormone Lab.

Address: Room 313, Bldg. 306

BARC-East

Beltsville, MD 20705

CRIS #: 1275-22000-081-00D

Telephone No.: 301-504-9396

FAX #: 301-504-8190

A. Research Accomplishments (up to 5) in Last Five Years:

1. Discovered a two-step regulation of decline of sex pheromone in mated females.
2. Discovered that high ambient temperature inhibited pheromone production in wild and laboratory reared females.
3. Determined the physiological basis for sterilizing effects of constant light in males.
4. Discovered and elucidated the daily rhythm of sperm release from testes.
5. Determined changes in protein pattern associated with maturation of sperm release mechanism.

B. Research Objectives for Next Five Years (brief description):

1. Elucidate regulation of sex pheromone production in females, in particular involvement of PBAN, and effect of temperature cycles.
2. Determine molecular basis of sperm release mechanism and post-testicular sperm maturation in males.

1. Purpose:

To understand the regulatory mechanisms controlling reproduction in gypsy moth males and females.

2. Significance:

An understanding of the reproductive physiology of males and females can provide the basis for the development of techniques to disrupt reproduction through novel approaches.

3. Constraints:

The two visiting scientists working in this area are on temporary basis. In order to continue this very productive research, at least one permanent position is highly desirable.

C. Current and Future Cooperators (ARS and Others):

Dr. J. Giebultowicz - University of Maryland, CP

Dr. J. Riemann - ARS, Fargo, ND

Dr. J. Joy - National Institute of Mental Health

Dr. B.S. Thyagaraja - University of Maryland, CP

D. Potential Uses of Research Findings:

1. Knowledge about reproductive physiology particularly mode of action of PBAN and control of sperm release will provide the basis for the design of novel control approaches.

2. Study of the effect of various temperatures on pheromone production can help in prognosis of the spread of gypsy moth into southern states.

E. Thoughts on Research Needs (not being addressed in other agencies or at State level):

Understanding of the reproductive physiology at the tissue and gene level is very important. It is only after this knowledge is gained that novel methods to disrupt mating and reproduction can be developed.

APPENDIX D. Systematic Entomology Laboratory

ARS GYPSY MOTH PROGRAM

Name: Douglas C. Ferguson

Laboratory: Systematic Entomology Laboratory

Address: c/o U.S. National Museum of Natural History
Washington, DC 20560

CRIS #: 1275-22000-049-00D

Telephone No.: 202-382-1777

FAX #: NA

A. Research Accomplishments (up to 5) in Last Five Years:

1. Conducted study of long-range dispersal of all migratory moths in eastern N. America. Was first to use Bermuda as a natural laboratory for this purpose. (Ent. Soc. Canada Mem. 158, 105 pp. 1991). Part still to be published.
2. Reported previously undocumented species (11) in U.S. & Canada (5 papers).
3. Solved other taxonomic or identification problems (9 papers).
4. Continued investigation of spanworm moths of tribe Semiothisini for Moths of North America series. Text for 140 species completed.
5. The laboratory previously revised and published the 37 species of Lymantriidae in the U.S. and Canada, established the correct generic name for the gypsy moth (Lymantria vs. Porthetria), and worked out its complex world synonymy (75 names).

B. Research Objectives for Next Five Years (brief description):

Completion or continuation of above projects and reexamination of the systematics of the gypsy moth complex in a broader context, including the Asian Gypsy Moth and its Old World relatives, of which there are thought to be about 10 species. The Systematic Entomology Laboratory has contracted with Dr. Yuri Tshistyakov, a Russian systematist at the Far East Science Institute, Vladivostock, to assist this project for one year by supplying specimens and information based on research in that region. Although all promising leads will be considered, the planned approach will be mainly morphological, concentrating on the male and female genitalia as character systems, and using specialized microscopical techniques not otherwise proposed as part of the Gypsy Moth Program. Related approaches might include comparative scanning electron microscopy or analysis and illustration of genitalic morphology by a combination of photographic and computer imaging techniques. How far this research can be carried depends upon funding, and a request has been made to APHIS for assistance.

1. Purpose and significance: Mostly self-evident. My research in general is to establish what species of moths live in North America, what they do, what they feed on, what they are related to, and how to identify them. No one else is doing this on the comprehensive scale necessary for the groups that I work on, because there are no other professionally employed specialists on these groups in the Western Hemisphere. With respect to the gypsy moth, all related species pose a threat, and foreign members of the group are poorly understood. It would be helpful to know and recognize them before they are introduced.

2. Constraints: No qualified technical support and very limited funding.

C. Current and Future Cooperators (ARS and Others):

Dr. Yuri Tshistyakov, Far East Institute of Biology and Pedology, Vladivostock.

D. Potential Uses of Research Findings: NA

E. Thoughts on Research Needs (not being addressed in other agencies or at State level):

One of the most important things that could be done would be to prevail upon the manufacturers of electric lights to develop and market a lamp that does not attract nocturnal insects--a lamp suitable for floodlighting and street lighting. It should be required that such lamps be used in foreign ports where ships and aircraft bound for the U.S. are loaded (or else require that they not be loaded at night in the warm season, which is probably unenforceable). Foreign countries could be given whatever assistance is needed for compliance.

Development of such an improved lamp would offer a double bonus. The mercury vapor lamp, of which millions are now in use for night illumination across America, is the most powerful insect attractant readily available, and it is probably so damaging to beneficial and harmless insects (most species) as to pose a serious environmental threat through the long-term decline of insectivorous birds, bats, small freshwater fish, and other dependent predators that we would not want to lose. This is difficult and costly to prove, but I am convinced that it is one more environmental catastrophe waiting to be discovered. The gypsy moth itself happens to be immune to lights because females do not fly, and males are active mainly by day (i.e., the European form).

I am not sure whose "mission" this is, but it would be to the credit of ARS to show awareness of the problem by advocating or promoting the development and use of a "low impact" lamp that would help to slow the introduction of foreign pests, as well as provide a better option for night illumination in general. The mercury vapor lamp, illuminating farms, barnyards, roadways, rural intersections, business sites, towns and villages everywhere, must be phased out. The sodium lamp, already also in common use, is a partial answer, but it still attracts about half as many insects as a mercury vapor lamp. There is a question as to how much more improvement is possible. This needs to be investigated.

ARS GYPSY MOTH PROGRAM

Name: Robert W. Poole

Laboratory: Systematic Entomology Laboratory

Address: c/o Natl. Mus. Nat. Hist. NHB-168

Washington, D.C. 20560

CRIS #: 1275-22000-049-00D

Telephone No.: 202-382-1786

FAX #:

A. Research Accomplishments (up to 5) in Last Five Years:

On gypsy moth - none

B. Research Objectives for Next Five Years (brief description):

On Lymantriidae - Systematic Catalog of the Lymantriidae of the World.

1. Purpose: To provide the basic Systematic information on the family Lymantriidae to which the gypsy moth belongs.

2. Significance: as above

3. Constraints: Primarily a Noctuidae Investigator, Lymantriidae Taxonomy being done on own time.

C. Current and Future Cooperators (ARS and Others):

None

D. Potential Uses of Research Findings:

Workers dealing with any aspect of the Lymantriidae.

E. Thoughts on Research Needs (not being addressed in other agencies or at State level): No entry.

ARS GYPSY MOTH PROGRAM

Name: Robert W. Carlson

Laboratory: Systematic Entomology Laboratory

Address: c/o Natl. Mus. Nat. Hist. NHB-168

Washington, D.C. 20560

CRIS #: 1275-22000-047-00D

Telephone No.: 202-382-1786

FAX #:

A. Research Accomplishments (up to 5) in Last Five Years:

I do perform some service identifications of Ichneumonidae parasitic or hyperparasitic on the gypsy moth.

APPENDIX E. Beneficial Insects Introduction Research Unit

ARS GYPSY MOTH PROGRAM

Name: Roger W. Fuester

Laboratory: Beneficial Insects Introduction Unit

Address: USDA ARS NAA BIIR

501 South Chapel Street

Newark, DE 19713

CRIS #: 1926-22000-004-00D

Telephone No.: FTS: 487-6095; Comm.: 302-731-7330

FAX #: 302-737-6780

A. Research Accomplishments (up to 5) in Last Five Years:

1. Characterized the host age acceptability and suitability, oviposition behavior, reproductive success, sex ratio, and incidence of nonreproductive parasite-induced mortality by the recently established exotic pupal parasite, Coccygomimus disparis. These studies showed that this species could attack both prepupae and pupae of the gypsy moth, female gypsy moth prepupae were suboptimal for parasite development, sex ratio ranged 80-92% female, and 88% of all hosts attacked died.

2. In cooperation with New Jersey Department of Agriculture and an ARS Research Associate, conducted an anlysis of the data on egg, larval, and pupal parasitism taken over 19 years in New Jersey permanent study plots. These studies showed that the introduced parasites Ooencyrtus kuvanae (egg parasite), Cotesia Melanoscelus, Phobocampe unicincta, Parasetigena silvestris, Blepharipa pratensis (larval parasites), and Brachymeria intermedia (pupal parasite) all exhibited density dependent responses to populations of gypsy moth.

3. Monitored the abundance of gypsy moth and the introduced predator, Calosoma sycophanta, in southern New Jersey from 1982 to 1991. These studies showed that the predator population responded to changes in gypsy moth abundance in a density-dependent fashion; the relationship being direct for larvae, but delayed for adults. Predation of gypsy moth pupae by Calosoma larvae peaked at 40% in 1983, the year of peak defoliation, and averaged about 30% for the next four years while the gypsy moth population was declining.

4. Completed laboratory studies on the biology of candidate exotic larval parasites of the gypsy moth: Glyptapanteles flavicoxis, Meteorus pulchricornis, Casinaria arjuna, and Hyposoter lymantriae.

5. Analyzed incidence in mortality by invertebrate natural enemies on male and female gypsy moth pupae. These studies showed that the introduced chalcid, Brachymeria intermedia, consistently killed higher percentages of male gypsy moth pupae than female pupae. Parasitic Diptera did likewise in 3 of the 9 years data were taken. Larvae of the introduced predator Calosoma sycophanta showed no such bias. Searches for biological control agents should focus on species that attack primarily female immature stages of the gypsy moth.

Research Objectives for Next Five Years (brief description):

To quantitatively characterize the role of parasites and predators in the population dynamics of the gypsy moth in forest environments.

1. Purpose: To identify the most important natural enemies, determine ecological factors affecting their efficacy, and to detect gaps in the natural enemy complex.

2. Significance: This knowledge would enable us to identify species which might be suitable for augmentative releases, suggest strategies for their conservation, and establish priorities for future importations.

3. Constraints: Adequate techniques are not available for accurately measuring the abundance of gypsy moth at low densities. When gypsy moth populations are low, it is difficult to obtain host samples of sufficient size to estimate parasitization.

In cooperation with ARS overseas biological control laboratories, to import high priority natural enemies and to develop technologies for their successful release and establishment in the United States.

1. Purpose: Permanent establishment of additional natural enemies would contribute to a long-term solution of the gypsy moth problem.

2. Significance: As the gypsy moth spreads, established natural enemies might not do well in new habitats. Exotic species which failed in earlier establishment attempts might do well in such situations.

3. Constraints: Host specificity studies needed to obtain regulatory permission to release "new" natural enemies will slow down progress.

In cooperation with Delaware State College and other collaborators, to monitor population buildup, dispersal, and impact of Coccygomimus disparis and other established natural enemies of gypsy moth in both urban/suburban and forest settings.

1. Purpose: To determine whether introduced species will be a causative factor in reducing gypsy moth populations.

2. Significance: By building on several years of baseline data, it will be possible to show a long-term trend in parasitism/predation. By working in both urban/suburan and forest settings, we will have a better grasp of the overall situation.

3. Constraints: None.

C. Current and Future Cooperators (ARS and Others):

Franck Herard & Herfried Hoyer (Ret.), ARS European Biological Control Laboratory, Montpellier, France, will cooperate in the importation of gypsy moth natural enemies from Europe, North Africa, and the Middle East.

Robert W. Pemberton, Research Leader, ARS Asian Parasite Laboratory, Seoul, Korea, will cooperate in the importation of gypsy moth natural enemies from Asia.

Paul W. Schaefer, Philip B. Taylor, Lawrence R. Ertle, & Kenneth S. Swan, ARS Beneficial Insects Introduction Research Unit, Newark, DE, will cooperate in one or more lines of research.

Robert Schroder, ARS Insect Biocontrol Laboratory, Beltsville, MD, will cooperate in studies on alternate hosts and host specificity of parasites.

Victor Mastro, APHIS Otis Methods Development Center, Bldg. 1398, Otis ANGC, MA 02542, will cooperate in the large-scale rearing of promising natural enemies.

Norman Dill, Department of Agriculture and Natural Resources, Delaware State College, Dover, DE 19901, will serve as the state cooperator for natural enemy releases in Delaware.

Ronald Priest, Michigan Department of Agriculture, Ottawa Bldg. Box 30017, Lansing, MI 48909, will serve as the state cooperator for natural enemy releases in Michigan.

Robert Chianese, New Jersey Department of Agriculture, CN 330, Trenton, New Jersey 08625, will serve as the state cooperator for natural enemy releases in New Jersey.

Richard McDonald, North Carolina Department of Agriculture, P.O. Box 27647, Raleigh, NC 27611, will serve as the state cooperator for natural enemy releases in North Carolina.

Edward Simons, Pennsylvanis Department of Environmental Resources, 34 Airport Drive, Middletown, Pennsylvania 17057-5021, will serve as the state cooperator for natural enemy releases in Pennsylvania.

John Tate, Virginia Department of Agriculture & Consumer Services, P.O. Box 1163, Richmond, Virginia 23209, will serve as the state cooperator for natural enemy releases in Virginia.

D. Potential Uses of Research Findings:

An improved understanding of the role of parasites and predators in gypsy moth population dynamics during the latent and progradation phases of the gypsy moth gradation could result in the development of better IPM strategies for dealing with building pest populations. Knowledge gained in studies on individual species could result in the development of augmentation or conservation strategies for improving natural enemy effectiveness. Up-to-now, efforts to use augmentative releases of parasites or predators against the gypsy moth have proved unsuccessful.

Because introduction of additional natural enemies generally improves the overall control of a pest, the addition of more effective exotic natural enemies of the gypsy moth to the existing parasite-predator complex would be beneficial. Further research on the recently introduced C. disparis may indicate that it is well-suited to the urban/suburban situation, because polyphagous, K-selected species often do better than specialized, r-selected species in disturbed habitats.

Since 1980, defoliation by gypsy moth in the U.S. has averaged over 4 million acres per year. It is likely that this figure will continue to increase as the gypsy moth incrases in range. Traditional suppression practices are not economically feasible over such large areas. Gypsy moth damage is of such magnitude that even a reduction of only 20% in suppression costs would result in annual savings of $2-3 million based on expenditures over the past decade. In addition, there would be savings in costs of removing dead trees, increment loss in plantations, and indirect environmental benefits due to reduced use of insecticides. Moreover, recent court decisions and economic considerations may dictate that greater emphasis be placed on development of biological control methods for gypsy moth, particularly those which involve the use of parasites and predators that actively seek out and destroy the pest.

E. Thoughts on Research Needs (not being addressed in other agencies or at State level):

1. Colonization of Established Natural Enemies over Generally Infested Area. This matter is not being adequately addressed. At least one important natural enemy, Calosoma sycophanta, lags behind the leading edge, possibly by several hundred miles. None of the states along the leading edge have a monitoring program in place, so it is even possible that other species need to be redistributed.

2. Development of Mass Rearing Techniques for Natural Enemies of Gypsy Moth. None of the Federal action agencies are rearing and releasing natural enemies of the gypsy moth. Only two states (Pennsylvania and Virginia) are rearing and releasing gypsy moth parasites. Even in those instances where gypsy moth parasites are being mass reared, the effort is generally limited to species which are easy to rear. No work is being done on univoltine tachinids, which are notoriously difficult to rear. This is unfortunate, because the rate of establishment for univoltine species has been high, ca. 80%. Moreover, Parasetigena silvestris, one of the most important established natural enemies operating at low host densities (and thus having potential for mass release against gypsy moth populations that are only starting to build), is a univoltine tachinid.

ARS GYPSY MOTH PROGRAM

Name: Paul W. Schaefer

Laboratory: Beneficial Insects Introduction Res. Unit

Address: 501 S. Chapel St.

Newark, Delaware 19713

CRIS #: 1926-24000-001-00D

Telephone No.: 302-731-7330 FTS 487-6095

FAX #: 302-737-6780

A. Research Accomplishments (up to 5) in Last Five Years:

1. Produced annotated bibliography on GM in Japan and the orient.
2. Documented the egg parasitism in Japan and Korea.
3. Recorded history of release and establishment of Coccygomimus disparis in North America -- the first new parasitoid in nearly 50 years!
4. Documented aerial predation on adult male GM by Dolichovespula maculata in Maine.
5. Currently measuring impact of Calosoma sycophanta populations on GM in field.

B. Research Objectives for Next Five Years (brief description):

1. Clarify behavior, biology & impact of Calosoma sycophanta on GM populations.
2. Determine significance of female flight in Asian GM, compared to European form of GM. This being done using body weight, wing loading and wing area.
3. Confirm defensive secretion of GM larvae using parasitoid bioassay tests.
4. Perform foreign exploration for potential natural enemies, focusing on GM in the Orient, and also investigating closely related Lymantria spp.
5. Complete world list of parasitoids, predators and other natural enemies on GM.

1. Purpose:

   Of the above objectives, 1, 4 & 5 are designed to better understand the natural enemy complex, to import potentially useful species, and to determine the impact the established species are having on the GM population. Other 2 objectives provide fundamental insights in GM.

2. Significance: No entry.

3. Constraints:

   a. Lack of sufficient funding dollars for foreign exploration.

   b. Limited biocontrol material for evaluation and consideration for release.

C. Current and Future Cooperators (ARS and Others):

David E. Leonard (Univ. Mass., Amherst, MA)
Kathy Sheehan (USDA, FS, Hamden, CT)
Jeffrey Miller (Oregon State Univer., Corvallis, OR)
Thomas Wood (Univ. Delaware, Newark, DE)
William Steinner (USDA, ARS, Columbia, MO)
Ronald Weseloh (Conn. Agr. Exp. Stn., New Haven, CT)
William Wallner (USDA, FS, Hamden, CT)

D. Potential Uses of Research Findings:

Findings will be published and will also serve as building blocks on which future research will be based.

E. Thoughts on Research Needs (not being addressed in other agencies or at State levels):

1. We need to genetically characterize all natural enemies of GM. This will contribute greatly to understanding species limits and origins of beneficial species following establishment. Understanding the founder effect will result.

2. With the arrival of the Asian gypsy moth in the Pacific Northwest, we need to investigate the basic genetic differences in various populations of GM in Asia and Europe. Such investigations will result in genetic markers that can eventually be used to identify origins of invading populations. This will also contribute to our understanding of species limits for L. dispar and its closely related species. Encolsed draft proposal is written with this objective in mind.

APPENDIX F. Asian Parasite Laboratory

ARS GYPSY MOTH PROGRAM

Name: Robert W. Pemberton

Laboratory: Asian Parasite Laboratory (Seoul, Korea)

Address: c/o American Embassy

Unit #15550

APO AP 96205-0001, U.S.A.

CRIS #: 4045-22000-002-00D

Telephone No.: 822-963-6561

FAX #: 822-969-4239

A. Research Accomplishments (up to 5) in Last Five Years:

1. The natural enemy fauna of gypsy moth in northern South Korea has been identified and ranked as to importance.

2. Gypsy moth larvae collected from trees which had extrafloral nectaries on their leaves had higher rates of parasitism than larvae collected from trees without the glands.

3. Survey work was begun in China. In 1991, collections in Shandong revealed a fauna very similar to that of Korea.

B. Research Objectives for Next Five Years (brief description):

a. Survey milder areas of South Korea.
b. Conduct studies to aid the utilization of identified natural enemies.
c. Continue cooperative work in China to survey Lioaning, the upper Yangtze River Valley and other areas of China for gypsy moth natural enemies.
d. Begin cooperative work to survey the Russian far east for gypsy moth natural enemies.

1. Purpose:

The primary purpose is to identify and obtain natural enemies for use in the US.

2. Significance:

Natural enemies from warmer areas of Korea may be useful in the southern range of gypsy moth in the U.S. Only a limited amount of work has been done in China and none in Russia. Both regions have unique natural enemies of gypsy moth that could be useful in U.S. control efforts.

3. Constraints:

Currently this laboratory supports the China work from its limited budget. No money has been allocated for the Russian gypsy moth work so I hope to have aid from headquarters to cover a possible shortfall.

C. Current and Future Cooperators (ARS and Others):

Korean Forestry Institute, Seoul
Chinese Academy of Forestry, Beijing
Russia - Far East Center of the Russian Academy of Sciences, Vladidvostok or Russian Quarantine, Moscow.

D. Potential Uses of Research Findings:

1. Provide new biological control agents of gypsy moth for use in the U.S.

2. Provide ecological and biological information about the agents to aid in their uses.

E. Thoughts on Research Needs (not being addressed in other agencies or at State levels):

Financial support for the cooperative Asian gypsy moth program in China and the program I hope to establish in eastern Russia is very important.

APPENDIX G. European Biological Control Laboratory

ARS GYPSY MOTH PROGRAM

Name: Franck Herard and Lloyd Knutson

Laboratory: European Biological Control Lab.

Address: Montpellier, France

c/o American Embassy, Unit 21551

APO AE 09777

CRIS #: 4012-22000-007-00D

Telephone No.: 011-33-67-04-56-00

FAX #: 011-33-67-04-56-20

A. Research Accomplishments (up to 5) in Last Five Years:

Herard, F., Keller, M.A., Lewis, W.J. 1988a. Rearing Microplitis demolitor Wilkinson (Hymenoptera: Braconidae) in the laboratory for use in studies of semiochemicals mediated searching behavior. J. Entomol. Sci., 23, 105-111.

Herard, F., Keller, M.A., Lewis, W.J., Tumlinson, J.H. 1988b. Beneficial arthropod behavior mediated by airborne semiochemicals. III. Influence of age and experience on flight chamber responses of Microplitis demolitor Wilkinson. J. Chem. Ecol., 14, 1583-1596.

Herard, F., Keller, M.A., Lewis, W.J., Tumlinson, J.H. 1988c. Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of Microplitis demolitor Wilkinson. J. Chem. Ecol., 14, 1597-1606.

Herard, F., Prevost, G. 1992. Encapsulement de Diadegma armillata (Hym.: Ichneumonidae) dans les deux hotes Yponomeuta cagnagellus et Yponomeuta malinellus (Lep.: Yponomeutidae). In: XXII iemes journees des entomophagistes, ENSA.M-INRA, Montpellier, France, 17-18 Mars 1992.

B. Research Objectives for Next Five Years (brief description):

1. Purpose:

   a. Collection in western Europe, north Africa and central Asia and shipment of sufficient numbers of parasitoids and predators of Lymantria dispar (LD) to the USA (West Virginia, Virginia, North Carolina), to provide species which have not been released or released in low numbers, and species or biotypes which are adaptable to warmer climates of southern States.

b. Determine alternate hosts requirements by the braconids Glyptapanteles porthetriae, and Glyptapanteles liparidis, and by the tachinids Carcelia separata and Palexorista inconspicua in southern France.

c. Measure effect of host density on parasitization by Carcelia separata and Palexorista inconspicua.

d. Study host attack behavior and bioecology of the tachinids Blepharipa pratensis and Blepharipa schineri.

2. Significance: Reduce populations of LD in eastern States and stop or limit its spread to southeastern States.

3. Constraints: Possible occurence of low level host populations for several years, and impossibility of exposing or disseminating very high numbers of gypsy moth larvae in forests.

C. Current and Future Cooperators (ARS and Others):

R. Fuester, Beneficial Insects Introduction Research, Newark, DE.

R. Schroder, R. Ridgway, Insect Biocontrol Laboratory, Beltsville, MD.

J. Tate, Virginia Department of Agriculture and Consumer Services, Richmond, VA.

R. McDonald, North Carolina Department of Agriculture, Raleigh, NC.

D. Potential Uses of Research Findings:

1. Importation and establishment of larval parasitoids from southern France into southeastern USA.

2. Identification of alternate hosts for LD parasitoids in southern France to better understand difficulties of establishment in the U.S. by multivoltine parasitoids.

E. Thoughts on Research Needs (not being addressed in other agencies or at State level):

1. Comparison of host acceptance and host suitability between LD and alternate hosts for multivoltine LD parasitoids.

2. Measure search rate and heritability of search rate in various biotypes of LD parasitoids.

3. Determine natural enemy host recognition mechanisms, kairomones, in LD parasitoids. Subsequently, determine potential of implementing host finding mechanisms in LD population management.

VII. Abbreviations Used

ACNPV	- *Autographa california* nuclear polyhedrosis virus
ADODR	- Authorized Departmental Officer's Designated Representative
AGM	- Asian gypsy moth
AIPM	- Appalachian Integrated Pest Management Program
APHIS	- Animal & Plant Health Inspection Service
APS	- Abnormal performance syndrome
ARS	- Agricultural Research Service
BT, *B.t.*	- *Bacillus thuringiensis*
CPV	- Cytoplasmic polyhedrosis virus
CSRS	- Cooperative State Research Service
cDNA	- Complimentary desoxyribonucleic acid
DM	- *Dasychira meridionaus*
DSG	- Decision support guides
ELISA	- Enzyme-linked immunosorbent assay
ELS	- Early larval survey
EM	- Election microscope
ES	- Extension Service
F_1	- 1st filial generation
FB	- Fluorescent brightener
FS	- Forest Service
FY	- Fiscal year
GIS	- Geographic Information System
GM	- Gypsy moth
HPLC	- High pressure liquid chromatography
IPM	- Integrated Pest Management
JH	- Juvenile hormone

LC_{50}/LC_{90}	- Lethal concentration to kill 50%/90% of insects
LD	- Lymantria dispar (the gypsy moth)
LdMNPV, NPV	- Lymantria dispar multiple embedded nuclear polyhedrosis virus, nuclear polyhedrosis virus
LM	- Light microscope
LT_{50}	- Lethal time to kill 50% of insects
MAB	- Monoclonal antibody
MOA	- Mode of action
NAGM	- North American gypsy moth
NCE	- Non-cerebral ecdysiotropin
NPS	- National Program Staff
PBAN	- Pheromone biosythesis activating neurohormone
PCR	- Polymerase chain reaction
PRC	- People's Republic of China
PTTH	- Prothoracicotropic hormone
PVC	- Polyvinyl chloride
RIA	- Radioimmune assay
RLO	- Rickettsia-like organism
mRNA	- Messenger ribonucleic acid
SAES	- State Agricultural Experiment Station
TE	- Testis ecdysiotropin
USDA	- United States Department of Agriculture
UV	- Ultraviolet